Conference on

PROBLEMS OF AGING

Transactions of the Thirteenth Conference
February 5-6, 1951, New York, N. Y.

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PREFACE

THE THIRTEENTH Session of the Conference on Problems of Aging was held under adverse circumstances arising out of paralysis of national transportation, due to a strike of certain railway employees.

Thus, certain of the members and invited guests, including one session leader as well as the Chairman, were unable to be present. The Conference is indebted to Dr. Clive McCay and Mr. Clark Tibbetts for their willingness to take the place of the absent program leader at very short notice

The Thirteenth Session was planned to serve as a second of a series of related sessions The Twelfth Session dealt with the problems of aging at the psychological-social level of functioning. The manifestations at the organ-system level were next to be considered; this year the endocrine, cardiovascular, and integumentary aspects being the focus of attention. Other topics at the organ-system level that likewise demand attention are other parts of the endocrine, the skeletal-articular, the digestive, the respiratory, and the nervous systems. It may be possible subsequently to deal with underlying cellular and metabolic processes, as such Advantage was taken of the presence in this country of Dr W. Jacobson of Cambridge, England, to anticipate in part the third phase of the series and to learn of his brilliant work on cell mitosis

ROY G. HOSKINS

Waban, Mass

Chairman

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Thirteenth Conference on Problems of Aging

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There is usually a nucleus of members who come regularly every year, and in addition there are guests who are invited for one or more meetings for their special contributions The setup of the meeting is not for the presentation of formal papers, but for informal and free discussion. The main purpose of the gathering is for the interchange of ideas, the back-and-forth discussion between those present, for mutual stimulation and for communication between the disciplines I am sure you are all to some extent aware of the difficulty of communication between disciplines, even such disciplines as anatomy and physiology.

But when you begin to deal with a problem—and we have in this area such a problem—which crosses all the way from the physical and biological sciences to the psychological and social sciences, then you are faced with the very great difficulty of communication between physical and biological sciences and social sciences

In a sense, we feel that medicine is in the key position to promote better communication Today medicine must be well versed in nuclear physics, because of the tracer techniques and the injury that can come from radiation, for example, and, on the other hand, medicine is certainly a social science and must be concerned with public health and, through mental health, with problems of social and economic importance In the concept of psychosomatic medicine, where we see psychological and social factors changing the functions of organs and organ systems and enzyme activity of cells, we begin to see the absolute necessity for understanding and communication between the social scientist and the enzyme chemist At the present time, we really have nature pretty much fragmented by our disciplines Universities set up specialized departments with walls between them.

We have discovered that it is very much easier for a representative of one discipline to communicate with a representative of quite a different discipline if they came from different universities You might think that over, but it is really true. Therefore we have found it to be a great advantage to select participants from different universities, from different disciplines Obviously, with twenty-five as a maximum num-

JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

FRANK FREMONT-SMITH
Medical Director

I WANT to welcome you all, and I want especially to say a word or two for our guests, to get them oriented as to the nature of these meetings. For a good many years now, the Foundation has been impressed with the need for several things that seem to be lacking in scientific research. One of them is the need for a multiprofessional approach, an exchange of ideas between representatives of the various disciplines which are concerned with a common topic. This particular field of aging is a nice example.

The second need is for a different kind of scientific meeting. Ordinarily, a group of scientists concerned with one discipline meets to discuss their problems; they do so by making formal presentations which are followed by two or three minutes, or sometimes as much as five minutes of discussion. The paper is presented in such a way that the author's main purpose in presenting it is to tie it up so that anyone who raises a question is really showing himself up and the author then is able to snap off the answers to those questions very quickly when he sums up the discussion I am overstating the case a little, but the point is that that kind of meeting is essentially a place where one makes statements *at* people and challenges them to dispute those statements. The actual need, we feel, in science, is much more for informal discussion, the kind which takes place in your own laboratories, where you sit around and tell each other not the beautiful things you have accomplished but, rather, your headaches and difficulties.

The story of how this all began, really, is centered in two men present today, Mr. Lawrence K Frank and Dr. Edmund V Cowdry. In 1936, Dr. Cowdry suggested that the Foundation should assist him in preparing a volume on problems of aging. Mr. Frank, who was then with the Foundation, said, "How can a group of scientists write a coordinated book unless they have a chance to meet and exchange ideas?" As the result of that suggestion, the Foundation held a two-day meeting at Falmouth, Massachusetts, in June, 1937, consisting of

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what frequently happens is that you begin with a hypothesis which may or may not be correct You test it; perhaps you get a hunch or a lead from someone else; you may make two or three false starts before your research gets under way Consequently, the final results may have little connection with the original idea. We use logic to test our hunches and our results But if we present only the logical end, we deprive our audience of a share in the interesting and exciting aspects of our research experience

If science is presented as a procedure of purest logic, there will be a tendency on the part of nonscientific administrators to feel that science, being logical, can be managed; that it can be controlled; that it can be ordered And there is nothing in our public relations, which I think are very poor, to counteract this impression. No one in a free country would think of ordering an artist to paint a landscape in a particular way I think the artistic aspects of science are as vital as the logical aspects We feel that in some small way the give and take in these informal discussions, preserved in these transactions, will give both sides of the process of scientific thought Therefore, we hope that you will talk about your headaches, your difficulties, and that you will even have *hunches* and express them in these meetings

The *transactions* are secondary to the discussions. They are the tail and they must not wag the dog The dog is what actually happens here among you

We think that Dale Carnegie really had something in the title of his book, *How to Make Friends and Influence People.* We feel you will be able to communicate with each other only when you have established a relationship of confidence, a friendly relationship Therefore, as part of the process of communication we want you to have the chance to associate with one another closely for the two-day period. We have seen people's work modified; we have seen spontaneous collaboration take place between different universities, between members of a conference, collaboration *not ordered* by anyone from above but coming out of a mutual need to approach a problem from several different angles simultaneously.

I will now turn the meeting over to Dr McCay

ber we can bring to a meeting, we must leave out a vast majority of the key people in every field; if we have seven or eight disciplines represented by three or four people—and in any important field there are going to be twenty-five to seventy-five key people—we must face the fact that we cannot include everybody. The smaller the group, the better the communication. We have played back and forth with this process and have found that a group of twenty-five people for a two-day period is the optimum.

I always like my chance to get in these introductory remarks, which must echo and re-echo in the ears of the members because they have heard them said so often. This has to do with the transactions of the Conferences. You see a stenotypist here, taking down everything you say. Well, that is not as bad as it seems, because you will get a transcript and you will have the opportunity to cross out anything that you wish you hadn't said or at least don't want to have in print. Therefore, we want to encourage you to speak with absolute freedom, complete informality, and without inhibition. Don't do what one man did—he crossed out everything he said. We prefer not to have that happen. We also would like to say: don't hesitate to ask seemingly foolish questions because how can you be sure they won't evoke wisdom in somebody else? It is the evocation of wisdom rather than the statements we make with which we are concerned.

This, then, is an experiment in communication. Each of our conferences has an immediate purpose—to advance research in a particular area. We like you to feel that you are a part of that experiment, that you contribute to it not only by your efforts at communication but also by giving us some wisdom. Will you send us your comments afterwards as to what can be done to better this communicative process? You all have in front of you the volume from the last meeting, and you will see that a good deal of the informality at that meeting has been preserved in the publication. We want to share what we are doing here as much as possible through the transactions.

Not only are scientific meetings somewhat stereotyped, but, far worse, scientific publications are very seriously stereotyped; they are in an editorial strait jacket. I hope if there are any editors here they will bear with me and be somewhat tolerant of what I am saying. I do not think there is any question but that there is perversion of the truth in the majority of our scientific publications. I mean that we have presented to the public and to each other only the logical rearrangement of our research work. Research is not always logical. It has a logical end; it always has, but its essence is the creative part. The creative is editorially censored, and we are forced to rearrange what we actually do in the laboratory and put it into a logical sequence. In research

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ENDOCRINE ASPECTS OF AGING

EARL T. ENGLE
Department of Anatomy, College of Physicians and Surgeons Columbia University

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Of course, you can do a thousand rats under completely controlled conditions, while it is extraordinarily difficult to do that on ten patients, but, on the other hand, those of us who are interested in the problems of medicine, particularly pathology, must do what we can to learn from the material which is presented to us from the experiments in the human which nature has set up for us It is only in that way that we are going to get at the fundamental nature of the biology of man and of woman

Also, when Roy Hoskins asked me to open the discussion, he knew that I was going to talk about the gonads; about the ovary and the testis and their hormones. The minute we get over into the thyroid and the adrenal we will call on other experts who are here

There are so many interesting things in the life history of the ovary and of the testis and their respective gamates that even at the risk of boring you, I thought I would run over some of these problems My thinking in this direction has developed in the last decade, after having gone through the whole series of laboratory animals, including the monkey, and then having been fortunate enough in the last decade to have had a great deal of experience with surgical material relating to

INTRODUCTORY REMARKS

CLIVE M. McCAY
Department of Nutrition, Stocking Hall
Cornell University

SINCE THE transportation difficulties have delayed the arrival of our Chairman, Dr. Hoskins, as well as other members from the northeast, I have been asked to serve as Chairman, *pro tem.* As you recall, it was decided to arrange a series of our meetings on problems of aging at different levels of complexity and integration. Last year our meeting dealt with the psychological, social, and economic problems of aging man. The meeting for this year was planned to focus attention on aging in certain organ systems. Obviously the list of organ systems is too extensive for detailed consideration in a single conference, so we have selected for emphasis the endocrine system, the cardiovascular system, and the integumentary system

I think I express the viewpoint of everyone here when I say that I believe nothing has contributed more to the stimulation of research cooperation than has the conference group of the Macy Foundation.

Dr. Engle will open the discussion on some of the endocrine aspects of aging.

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Department of Anatomy, College of Physicians and Surgeons
Columbia University

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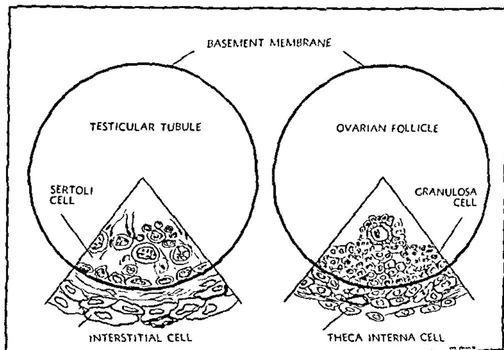


FIGURE 1 Schematic illustration which indicates essential homology between the testis tubule and the ovarian follicle. Within the basement membrane of the two structures is the gametogenic function of the two gonads Outside of the basement membrane is the site of elaboration of the respective steroid hormones The Sertoli cells, in this concept are homologies of the granulosa cells, both supporting or nursing cells. Gametogenic function of either is lost with the destruction of the basement membrane

the human ovary and testis and their hormonal products Therefore, I want to lay some of this fundamental groundwork, hoping that you will interrupt my presentation as thoughts occur to you

We will begin with a single unit. The human gonads, the ovary and the testis, are so designed that they have two major groups of products, one being the germ cells for the perpetuation of the race, and the other a peculiar group of steroid hormones which are certainly essential to the reproduction of the race and, in a larger way, have many physiological activities in the general somatic economy.

I did not bring lantern slides because I think they more or less freeze the conversation, and I wanted to get some comments in general This figure (Figure 1) shows a unit of the testis, and of the ovary The characteristic homolog of both of these structures is this membrane

which in the testis is called the basement membrane and which in the ovary is the hyaline membrane, although they are both, so far as I can find out, precisely the same thing. They were simply historically first observed and described by different individuals.

Within this area of what we are now going to call the ovarian follicle is a group of polygonal, low columnar cells which are known as the granulosa cells. This is a mature follicle, bearing an egg, and we are going back to the origin of this egg in a minute, because it is important to the thesis The whole function of this follicle is to protect and nourish that egg until it is ready to undergo reduction division and be extruded from the follicle and be susceptible as a gamete, ready for fertilization.

In the testis, on the other hand, we have a group of cells called Sertoli cells The earlier cells differentiate in the embryo, and they are the last cells to disappear in old age They have a perfectly characteristic nucleus This is the Sertoli cell Then we have a whole series of cells in the testis, the spermatogonia, the primary spermatocyte, the secondary spermatocyte, the spermatid and then the spermatozoa, or the mature sperm cell The whole function of these germ cells is to carry on through successive myriads of generations in the production of vast, astronomical numbers of sperm.

The Sertoli cell is quite a different cell, and while we don't know very much about its internal chemistry, we do know that it is necessary, within this tubule, for a certain state of the maturation of the sperm cell It is a peculiar cell because, so far as anybody has been able to demonstrate, there is no apical membrane Dr Heilbrunn, I hope, will tell us something more about the potentialities of cellular physiology in which you can have a cell which has no definite ectoplasmic membrane around an apex

The reason why nobody has ever been able to demonstrate any membrane here is that it is a free-flowing colloidal cytoplasm at the apex, which is moving and changing within the tubule. As sperm develop and the spermatid is formed and begins to go through its normal process of shucking off cytoplasm, it is taken up by this Sertoli cell and is held there for a certain period of ripening There are many things we don't know about it, and so we will just have to leave it because it has to do with fertility and not with aging

Now, the other thing which you must keep in mind is that within these two membranes of the ovarian follicle and the testis tubule, the sole function is the production of normal gametes There are no capillaries within this membrane All exchange of gas or metabolites must go through this intact basement membrane. The other thing—and I

am sorry that Fred Hisaw isn't here because I would get an argument right now—is that there is no known hormone production going on in these cells within the membrane. We will come back to the question of the possibility of production of estrogens by the Sertoli cell, but it is still speculative and unproven.

In the past, we have been taught, from the time of Edgar Allen on down, that the steroid estrogens were produced by the granulosa cell within the follicle. None of us can substantiate that when we use current cytochemical methods for the demonstration of phospholipids and cholesterol, and the rather inadequate methods for demonstration of ketosteroids.

Outside this basement membrane in the ovarian follicle there are polygonal cells, in adult life, which are known as the *theca interna*, which have an interesting life history, and therein they would be associated with these capillaries. Capillaries are woven in and out and around. So far as we can find in the mammalian ovary and certainly in the human ovary, steroid production occurs within these cells. Outside of the testis tubule are also islands or groups of cells, which give all the cytochemical reactions for lipids and ketones and are the well-known cells of Leydig or the interstitial cells

Here, again, you see, we have a number of regulating factors. We have, within this type of membrane, areas for the production of sperm in one and of eggs in the other. They are completely independent of the rest of the gonad structure Outside of that, we have typical parenchymal cells whose prime function is the production of one or more of a group of steroid hormones

This we also know, that as soon as the integrity of this basement membrane is lost, gamete production falls off immediately The first indication that atresia is taking place is when the basement membrane becomes pervious, and capillaries and phagocytes begin to migrate. The first sign we have that sperm production is going to fall off is when this basement membrane and its associated *tunica propria* become thickened and fibrotic, thereby pushing the capillaries farther and farther away, thereby setting up a greater physical barrier for the passage of gas and metabolites. We therefore have two units in the human gonad that protect the gamete on the one hand and, on the other, produce the steroid hormone complex needed for reproduction

Again, we have to go back to changing thoughts in biology, particularly on the ovary. It was originally thought, back in the '70's or '80's of the last century, that when the human infant was born at term, the ovary had all the egg cells it was ever going to have, and that those ova were used and distributed over the limited reproductive period of woman. From age fifteen to age forty-five, she would not liberate

more than 350 eggs. Then other men came along, working with mice and rats, and came out with the idea that there was a continuous pro- [illegible] pathologists accepted the idea that, of course, if this was true in the rat and the mouse, it must be true in the human, and that there was no restriction on the number of germ cells produced by the human ovary.

Let us assume that the human ovary at birth, which has a vast number of eggs in it—two hundred thousand or four hundred thousand eggs—if those are going to be replaced steadily throughout adolescence and reproductive life, then there ought to be pretty much of a uniform supply of ovarian eggs present whenever the ovaries are examined, which, as you all know, just is not true As you examine the ovary of the human newborn and at two years and five years and so on, the one thing that impresses you is the consistent and marked loss and destruction of eggs In a sense I think, with Professor George Minot, that in the human egg cell population the process of *aging and death* begins at birth

No one has had a chance to see a large number of normal ovaries Ovaries from children who have died of illness and so on are not pertinent to the subject, so almost all of my studies have been made on biopsies of the gonads in infants in which there was a question of sex determination We have found that the only way one can actually determine whether the genetic constitutional character of the individual is male or female is by doing biopsy studies of the gonads, and we have a fair amount of such material I therefore take the liberty of saying that in my experience, the loss of human eggs begins at or before birth It continues at a very considerable rate until adolescence—and here I am guessing because we have insufficient or no information, but I would suggest that if a human ovary had two hundred thousand eggs in it at birth, half of those will be gone by menarche and the time of first menstruation. The rest disappear rather rapidly. Then there comes a great difference in ovaries of [illegible] en who have had [illegible] It may be very [illegible] women, while ir [illegible] eggs at age fifty-five Therefore, the rate of loss of eggs (and I think this is fundamental to the aging process) begins to fall off before eggs are ever used for reproduction, and the rate of falling off shows a great deal of genetic and constitutional variation among different women

On the other hand, the human testis at birth has no cells which are differentiated; you cannot distinguish between the Sertoli cell and the spermatogonia. These spermatogonia migrate into the testis *anlage*, as do the eggs, early in embryonic life, according to the best that the embryologists can teach us. But in the human ovary, according to my concept, they are all present at birth. In the human testis, these cells are undifferentiated, but at about two or three years of age, you can begin to pick out a true Sertoli cell and at about the same time an occasional spermatogonia. Those spermatogonia must then divide, must continue to make a whole continuous series of cells leading up to the sperm.

Now, I was saying that the eggs are liberated during the limited period of reproductive life from fifteen to forty-five, with a plus or minus of five years. In the testis, germ cell production can and does go on throughout life, although the production of sperm does begin to fall off statistically around thirty-five years of age.

Now, let's look at some other things, such as hormone production. Wouldn't you like to argue a little bit about this idea that human granulosa cells don't make any steroids? Can't I start off a little argument here?

Hamilton: Not at the moment.

Engle: I'm sorry, because if Dr. Hisaw were here, we would really have an argument going.

Levin: Suppose I accommodate you, Dr Engle In my own mind, I still—

Engle: Let me say that Dr. Levin and I are old friends We worked for years side by side. Dr. Levin is a mouse man

Levin: All those gallons of urine that I used in my scientific lifetime did not come from mice. However, I am thinking of the original data of Doisy and his group (21), which is not yet adequately explained by the hypothesis you advanced. As you remember, Doisy's group was working with follicular fluid of the sow and in it they found concentrations of estrogens greater than in any other portion of the ovary As a matter of fact, the most potent of the natural estrogens (α-estradiol) is found in the follicular fluid.

Engle: That's right.

Levin: I don't see how you can explain the high concentration of estradiol, and also of estrone, in the follicular fluid if its presence there is only due to diffusion or something of that order. The fact remains that estrogen occurs in the follicular fluid in larger concentration than in other portions of the ovary and to me this would imply that it is actively secreted into the follicular fluid and, because of the granulosa

cells immediately surrounding the antrum, that these are the cells responsible for estrogen production

Engle: It may be that you are right, but I believe that cytochemical studies are continuing to provide the evidence for my present viewpoint.

Dr. Wislocki and his associate, Dr. Dean, have in press a paper on the lipids of the ovary of the rat and sow, and their observations show that the normal follicle has little or no lipid in the granulosa

As you look at any cross section of an ovary, I don't care whether it is mouse or human, most of the follicles that you see are atretic follicles; they are follicles with damage to the basement membrane; they are follicles which are losing granulosa cells; they are follicles in which the egg already gives signs of dying In our material, and also in Dr Dean's material on the sow, it is the atretic follicle that has the large masses of lipid and cholesterol within the granulosa and throughout As you know, for every egg that is liberated capable of fertilization, there are another dozen follicles that have come almost to maturity but have then undergone atresia without liberating an ovum. It may be that the biological purpose of building up all of these masses of follicles, when there are never going to be more than about three hundred follicles producing an egg that is potentially fertilizable, is to produce steroids In the atretic follicle the theca cells will line up radially and the granulosis cells pretty much disappear. At that stage the follicular fluid certainly is full of estrogen There is no question about it But I think it appears only in atretic follicles and not in normal follicles

Goldzieher May I interject at this point a question pertaining to the pathology of the ovary? The majority of estrogen producing ovarian tumors consist of cells which are not necessarily derived from granulosa cells although these growths are called *granulosa cell* [illegible]
type a[illegible]
the m[illegible]
tumor[illegible]
functi[illegible] do not conform to the ordinary structural pattern of granulosa cell tumors, they do produce estrogens.

Levin: In what sense do you mean "follicular" but without the usual granulosa cells?

Goldzieher: They contain follicles [illegible]
[illegible]
[illegible] and
[illegible] name for they are more likely to be derived from *theca externa* or stroma cells than from the granulosa cells of the ovarian follicles

Engle: I am glad you brought that up, because otherwise I could not properly have gone into this field of ovarian tumors, which is highly specialized. I think you are quite correct. The bulk of the granulosa cell tumors are hormonally silent tumors. They produce no steroids. In our experience, and that of Hertig in Boston, if you take any of these granulosa cell tumors and do lipid stains throughout, you will find that the bulk of the granulosa cell tumor has no lipid in it and is probably not producing steroid but throughout the tumor you will find islands of luteoid cells and they are rich in lipid. One does not see many granulosa tumors. But in my experience, I have never seen one that was hormonally active that did not have enlarged islands of luteoid cells which were heavy in lipid.

I think that is an argument that may stand up. The typical granulosa cells, which are composed of cells like these, are hormonally silent, and it is only when you get the luteoid areas that you get hormonally active cells. We need more material, of course.

Levin: There is one more point. If this whole thesis is correct, you are confronted with the necessity for an explanation of the results one gets by treating animals (and I think it has been done in humans, too) with certain gonadotrophins One can thus induce considerable estrogen secretion, as indicated by all the usual signs, and yet not find the slightest indication of luteinization

Engle: You are talking of the work with so-called purified pituitary gonadotrophins and so on?

Levin: Yes

Engle: But you don't. Purified follicle stimulating hormone (FSH) is not active unless you drop a little of the luteinizing hormone (LH) in it, and that is active on theca cells only.

Levin: I know. That is correct. But histologically there is no sign of lutein change We can also consider the case in which Kurzrok and Smith (14) administered the urinary gonadotrophin of postmenopausal women to human female subjects and observed the production of cystic follicles with estrogen secretion but no signs of luteinization.

Engle: That's right, cystic follicles are always atretic follicles They have lost most of their granulosa cells and the only functional cell remaining is the theca cell.

Levin: I think they used a preparation from postmenopausal urine but regardless of source the point is, there was estrogen secretion without lutein change

Engle: Well, I want to get back to my main thesis on the fundamental differences between the aging of the human ovary and testis What I am saying, in essence, is that this egg-cell-producing unit in

the ovary, by and large, does not have too much to do with producing the steroids. As one gets toward the older age groups, the decrease in the number of egg cells also leads to a decreasing number of follicles, so that in woman, at the end of reproductive life at age forty-five, there are no more growing eggs, and the number of growing follicles is getting fewer, so that finally, when menopause occurs, all the eggs are gone Then, of course, the ovary becomes inert from the standpoint of estrogen production because in the ovary—and I think this is true of the mouse as well as woman—the presence of eggs is necessary for the production of steroids. You have to accept that. Well, it isn't true of the mouse, either, because A. S Parkes showed a good many years ago that you could irradiate the ovary of the mouse and get rid of all the follicles leaving a greater interstitial mass of lutein cells and the mouse will go right along having regular cycles indefinitely But in the human, I think that we have to consider that the presence of the egg and the atresia of the follicles after it reaches a considerable size are necessary for the production of the hormone, because after the eggs have all gone in this menopausal or premenopausal ovary, there are no more of any of the steroid-producing cells, and that is why in woman these changes occur and why menopause appears as a curtain which is drawn down at a given calendar year.

In the male, on the other hand, we know this is not true because the Leydig cells can produce hormone independently of the presence of sperm One can find in young men, as I have, far too many times, nothing but a ghost tubule, where that whole tubule is completely fibrosed yet their genital development and their sexual activity and production of 17-ketosteroids remain relatively unchanged. In other words, in woman, hormone production is dependent on the [illegible] development [illegible] p[illegible] o[illegible] [illegible] sperm production and the Leydig cells remain functional

Now, let us look at the problem again in two areas of pathology. We have in woman one of nature's own experiments which we know as ovarian agenesis You all know the clinical configuration of the girl who is brought into the clinic, somewhere between the time when she is fourteen and eighteen She is short of stature; she frequently has a web neck, she sometimes has coarctation of the aorta, and other congenital disabilities of that nature She appears infantile, and on those occasions in which laparotomy has been indicated, the ovarian cord can well be made out and, on section, that ovarian cord has characteristic ovarian stroma in it. But there are no egg cells. There never

were any egg cells. The primordial ovocytes should have made their migration from the genital ridge into the developing gonads, but just failed to arrive. This individual, throughout life, then, is, from the standpoint of her own ovarian steroids, a neutral individual. In the absence of eggs, no steroids are produced.

On the other hand, we have a syndrome in the male which has been given half a dozen names by now, which is characterized by perfectly normal tubules, with no germ cells. This tubule has nothing but Sertoli cells in it, very good, healthy Sertoli cells. It also has perfectly normal Leydig cells. It is found in early adolescence, and the bulk of the cases which we have occur in young adult life. The testicular pathology I have called germinal aplasia, which is about as noncommittal a thing as I could think of. To me, it means that, just as in ovarian agenesis, the germ cells did not make the migration during embryonic life.

You do find, in a good many pathological lesions, situations similar to this in which there are no germ cells, in which a differential diagnosis must be made But in most of them, whether it is due to some intercurrent infection or disease process, nutritional disability, or, of course, the most prominent, radiation effect, there is always a thickening of the basement membrane and the *tunica propria*. According to the definition of germinal aplasia, the basement membrane must be normal, the contour of the tubule must be uniform and regular, and the tubule must be of normal size.

Now, what is the difference in this boy and this girl, or in this adult man and this adult woman without germ cells? The hormone production is always completely normal so far as we can find out in the male. Some cases which have been reported do have a high FSH but certainly not all. They do have normal 17-ketosteroid excretion and normal genital development In women, however, there is complete lack of ovarian steroids

Thus, in woman, steroid hormone production is dependent on the presence of a succession of rapidly growing follicles; while in the male, the steroid hormone production goes on quite independently of the presence of the gametes

I came down here, planning to present data on the excretion of steroids at various age periods, and I was delighted when I saw my old friend, Jim Hamilton, walk into the room, because he and his associate have done one of the most thorough studies on the excretion of ketosteroids in relation to age Before we go any further I would like to have Dr. Hamilton give us the results of his studies At this stage, Dr. Hamilton, we want to know what the 17-ketosteroids indicate about production of gonadal steroids and what is their statistical pattern in relation to various decades of life.

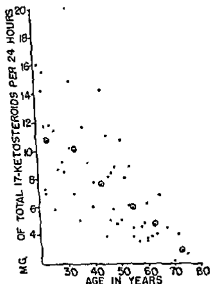


FIGURE 2 Age changes in 24-hour excretion of total 17-ketosteroids in adult males

Adapted from Hamilton, H and Hamilton, J Ageing in apparently normal men I Urinary titers of ketosteroids and of alpha-hydroxy and beta-hydroxy ketosteroids, *J clin Endocrinol* 8 433-452, 1948

Hamilton May I expand my answer to include more than ketosteroids?

Engle Oh, yes.

Hamilton: These data are for supposedly normal males (Figure 2). Anybody who has tried to get a group of supposedly normal people knows that "normal" should be in quotes. We made quite an effort to do that but we found we could not get supposedly normal men in hospitals or homes for the aged, so we went to prison—and got in and out each day successfully These men were put in there because they were bad and not because they were sick Even so, there is a great deal of question in our minds as to whether or not they should be termed "normal"

The curve of the *average excretion of ketosteroids decreases progressively* and materially with age (5). That is the handwriting on the wall for men

The first point I wish to make is that these values for ketosteroids are paralleled almost exactly by titers for urinary androgens in the same people, so that there is a similar curve for the decrease in urinary androgens.

The cardinal problem, in much of what we have been trying to do, is to quantitate some of these maleness aspects of aging, and we have turned to a third procedure; namely, the measurement of axillary hair (7). To some extent, we think of axillary hair as a rooster's comb; that is, as something that can be studied just as effectively as the masculine appendage in the fowl. The story on axillary hair follows a similar pattern; in fact, it has a correlation coefficient of about .6 with ketosteroids, which pleases us very much (7). For reasons which I do not wish to bring up now, we think that it may be related to titers of dehydroisoandrosterone in the urine. As far as the endocrine aspects are concerned, there is a material and definite decrease with age in all three characteristics that we are able to use for such testing. I don't know of any good studies yet on the relation of the aging processes to sperm analysis that could be compared with this.

Should I consider females in this discussion?

Engle: Oh, yes, do.

Hamilton: We have no data of our own on ketosteroid excretion among females but we do have values for axillary hair growth. First, though, I would like to present data for axillary hair in males according to age (Figure 3). Starting from values of zero at adolescence,

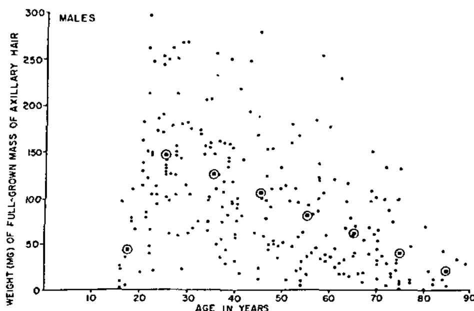


FIGURE 3.* Age changes in the weight (mg) of the full-grown mass of axillary hair in males from the second to ninth decade of life Points represent values for individual subjects Circled squares represent average values for each decade

*Figures 3 and 4 reprinted from Hamilton, J: Quantitative measurement of a secondary sex character axillary hair. *Ann N. Y. Acad Sci*, 53 585-599, 1951

the growth of axillary hair in males reaches a peak somewhere in the twenties to thirties, and thereafter decreases as do the values for urinary ketosteroids

We have used three ways to measure axillary hair. The first is the mass, or weight of a sample which has been treated to remove fat and lint and other contaminants The sample is also dessicated before weighing

The second feature we can use in measuring axillary hair is daily rate of growth. Since the mass and the rate of growth of hair are related, the amount of growth per day varies with the mass of hair It is a beautiful relationship. We therefore have come to use a ratio, involving both factors, which is the rate of growth divided by the mass of hair.

This diagram, then, relates to the mass of full-grown hair in males.

The next curve is for females (Figure 4). Females mature a little earlier, and their values are higher in early adolescence. The values reach only about two-thirds of those in males and then decrease a great

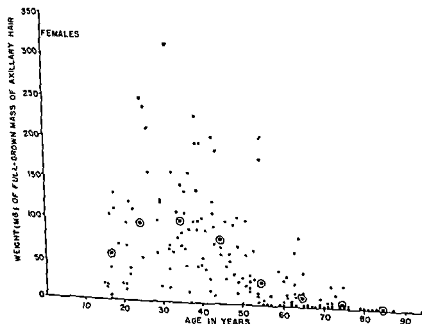


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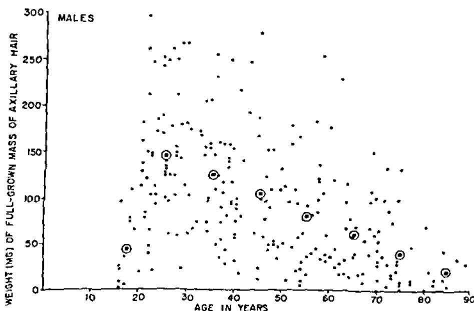


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or German They were mixtures of various genetic backgrounds.

Horvath. Are these women who never shave under their arms at all? That presents a difficult problem—the present female population being so concerned with their underarm appearance

Hamilton: Yes, there is quite a problem there. I suggest that people going ahead with this method think in terms of daily rate of growth in women, because they do tend to shave off hair.

Oliver: Does shaving stimulate the hair growth?

Hamilton: I can answer that One of the values of this method (7) is that one can take samples from intervals as short as a week or as long as a hundred days, and we have various intervals to see whether one could use, let's say, a week or six weeks or any other length of time interchangeably. The answer is that one can I am sure that it is possible that shaving might influence hair growth, if one evaluates it at daily intervals, but it doesn't make any difference whether one shaves every week for six months or takes one shaving now and another one six months later

Engle I think Mildred Trotter showed many years ago that shaving did not accelerate hair growth

Hamilton That's right Are there any other questions?

Steele: Does hair stop growing eventually or does it keep on? Does it fall out?

Hamilton With aging the area of skin covered by hair becomes more restricted The axillary hairs that still grow tend to be shorter, thinner and less kinky So there is atrophy of the actual hair and a loss in terms of distrib[illegible]

[illegible] there are a number of factors that influence the amount of growth that is attained in an individual First, there is a genetic factor Second, there is an age factor Third, there is a distinct gonadal factor, because the oophorectomized woman and the castrate man have almost identical values They are very low but almost identical Another factor is what we are calling "seasonal," which is a very poor term There are variations over the course of the year, and there are very wide swings, usually of about six months. There are changes from time to time that are not really related to seasons but, over the year, do show such changes (7).

Another factor that enters into this is the responsiveness of the individual If we treat eunuchs with a standard amount of male hormone (testosterone propionate), and inject them each day with the same amount of vehicle in the same site, they, too, show these time-to-time variations over a period of years of about the same extent as

deal, near and after the time of the menopause so that they tend to be close to zero by sixty years of age.

Oophorectomized women, instead of having average values of around 100 mg. in the third and fourth decade, have very low values, about as low as those in eunuchs.

Levin: Are you now referring to mass or to rate of growth?

Hamilton: Mass; weight of the full-grown mass, actually.

This same type of procedure would work on pubic hair, but the axillary hair forms a discrete mass that can be shaved and measured quantitatively.

To summarize, then, the change that goes on with age in the males occurs in the females but much earlier.

Engle: Do your data indicate a reduction in axillary hair in women after the age of forty?

Hamilton: In serial studies of some females a loss of axillary hair has been noticeable in the late thirties and in some instances can even be seen in the early thirties. This is not the rule.

Stern: Where did you take the old age group of females, from what type of people? Were they also prisoners, or what?

Hamilton: They were not prisoners They were people who lived in institutions, particularly institutions for the insane We have had to check that group with a supposedly normal group of medical students and personnel employed by a medical school There is no difference between our people outside the insane institutions and those inside the institutions, as regards values for axillary hair.

Oliver: But there are different sorts of men and women and some have more hair than others How do you take care of that?

Hamilton: You are absolutely right What I am quoting here are average values. These are averages per decade, as represented by the line I have drawn The values may vary between 0 and 300 milligrams.

Engle: How about variation when you break it down by racial origin?

Hamilton: That is very important

Oliver: It seems to me you start with a mixed lot of stuff and end up with one curve, an average of a mixed lot

Hamilton: Do you mean people of different ages?

Oliver: No, different sorts of people; some "hairy" and some not

Hamilton: We have had to construct different standards for Caucasians and people who are of Oriental race. The curve for the Chinese is entirely different from that for Caucasians.

Engle: How about Northern and Southern Europeans?

Hamilton: I can't answer the question on differences among Caucasians. That is quite a different problem. We did consider it in our series of prisoners, but we found almost none of them were English

or German They were mixtures of various genetic backgrounds.

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Sh. [illegible]

H [illegible] but there are a number of factors that influence the amount of growth that is attained in an individual First, there is a genetic factor. Second, there is an age *factor* Third, there is a distinct gonadal factor, because the oophorectomized woman and the castrate man have almost identical values. They are very low but almost identical Another factor is what we are calling "seasonal," which is a very poor term There are variations over the course of the year, and there are very wide swings, usually of about six months. There are changes from time to time that are not really *related* to seasons but, over the year, do show such changes (7)

Another factor that enters into this is the responsiveness of the individual If we treat eunuchs *with* a standard amount of male hormone (testosterone propionate), and inject them each day with the same amount of vehicle in the same site, they, too, show these time-to-time variations over a period of years of about the same extent as

deal, near and after the time of the menopause so that they tend to be close to zero by sixty years of age.

Oophorectomized women, instead of having average values of around 100 mg. in the third and fourth decade, have very low values, about as low as those in eunuchs.

Levin: Are you now referring to mass or to rate of growth?

Hamilton: Mass; weight of the full-grown mass, actually.

This same type of procedure would work on pubic hair, but the axillary hair forms a discrete mass that can be shaved and measured quantitatively.

To summarize, then, the change that goes on with age in the males occurs in the females but much earlier.

Engle: Do your data indicate a reduction in axillary hair in women after the age of forty?

Hamilton: In serial studies of some females a loss of axillary hair has been noticeable in the late thirties and in some instances can even be seen in the early thirties. This is not the rule.

Stern: Where did you take the old age group of females, from what type of people? Were they also prisoners, or what?

Hamilton: They were not prisoners They were people who lived in institutions, particularly institutions for the insane. We have had to check that group with a supposedly normal group of medical students and personnel employed by a medical school. There is no difference between our people outside the insane institutions and those inside the institutions, as regards values for axillary hair.

Oliver: But there are different sorts of men and women and some have more hair than others. How do you take care of that?

Hamilton: You are absolutely right What I am quoting here are average values. These are averages per decade, as represented by the line I have drawn. The values may vary between 0 and 300 milligrams.

Engle: How about variation when you break it down by racial origin?

Hamilton: That is very important.

Oliver: It seems to me you start with a mixed lot of stuff and end up with one curve; an average of a mixed lot

Hamilton: Do you mean people of different ages?

Oliver: No, different sorts of people; some "hairy" and some not.

Hamilton: We have had to construct different standards for Caucasians and people who are of Oriental race. The curve for the Chinese is entirely different from that for Caucasians

Engle: How about Northern and Southern Europeans?

Hamilton: I can't answer the question on differences among Caucasians. That is quite a different problem. We did consider it in our series of prisoners, but we found almost none of them were English

Dr. Cowdry is here now, and I think, as dean of the gerontologists, he should take over.

Cowdry: I think this kind of meeting proceeds best with a minimum of assistance. It just naturally flows on. I don't know what comes next, except to follow up your introduction about these very remarkable differences between the two sexes. I think you can consider it on the economic-psychological sphere and you can also consider it on many other levels. What is the program? How shall we proceed from now on? Do we have a psychiatrist here?

McCay: That was discussed in planning the program, too, and Dr. Stern is here.

Stern: I suggest that, first, perhaps, we continue listening to Dr. Engle before we get into the clinical-psychiatric features of aging.

Cowdry: We would be very happy to listen to Dr. Engle.

Engle: Well, gentlemen, you are asking for your own punishment because when it comes to this field, I can run along indefinitely. This sex difference in longevity is important. Clive McCay and I have talked about it a lot.

Now, there is another problem. I don't know why it is that woman, as she approaches or passes age thirty-five, is losing eggs and producing hormones at an accelerated rate. We don't know enough about the metabolic actions of either the estrogens or the androgens. There *is* one item that is seen constantly as you examine the ovaries, surgically removed in women of these older groups, and that is, there is a consistently increasing frequency of various types of vascular pathology. When one sees the ovary of a woman when she is still menstruating or has passed menstruation, a woman of forty to forty-five, the first thing that strikes the eye is the great degree of arterial and arteriolar damage. I should like very much to do some systematic work on the problem, but I never have the time. But in the ovarian hilum, the ovarian artery comes in as a definite structure and then it breaks up into a group of spiral arteries that feed out toward the cortex. If you make sections anywhere in the hilum in these older age women or in younger women who are having a good deal of disturbance which has necessitated pelvic surgery, there may be a thrombus formation in the ovarian artery before it breaks up into its spiral components. There may be evidence of thrombi which have been recanalized. There is very frequently evidence of cholesterol or calcium plaques.

Fremont-Smith: Is this in the main artery?

Engle: Yes, in the ovarian artery.

Fremont-Smith: Are you implying that for a while the artery had been actually completely obstructed before it was recanalized?

normal people do. So that is another variable in this measurement.

Steele: I hate to be persistent, but I can't get a picture of hair growing at the rate of 2 millimeters every 24 hours for six months and then, boom! stopping. Obviously, your axillary hair doesn't keep on growing down to your knees. What stops it? Isn't there a decline in rate of growth?

Hamilton: I think I see the question you are raising. First, the hair grows only so long and then it stops growing and, after a quiescent phase, drops out and another hair appears. It grows, actually, for about a six-month period. That is the length of life of a single axillary hair at the longest. Some have even shorter cycles. Does that answer your question?

Steele: Yes, that is it.

Hamilton: To get the weight of the full-grown mass of hair, we insist that no shaving be done for the past six months; otherwise, it is not a valid measure.

Steele: That answers it, then, because, obviously, if you took rate of growth at six months and again at a year, you would decide that it was growing twice as fast if you had taken the six months period instead of the year.

Hamilton: Oh, no, it comes back to reach its previous level in six months It does not grow beyond that

McCay: In continuing, it might be worth just a sentence to think of why Dr Hoskins set up this session with Dr. Engle leading off We thought we ought to give some attention to the problem of the difference between the sexes, in life span and aging. Over the years, we have constantly talked about this whole matter of why the female of the species outlives the male, and we have discussed here many times the problems of sociology and the economics of having a great group of old women burying us men We have discussed the problem of why the female rat always survives the male That is the reason for the arrangement of the program. We are trying to answer the question of why the female outlives the male. What does it go back to? From the point of view of sociology and economics, the greater longevity of the female has tremendous importance. We have the many basic observations in other animals and also the many beliefs that Dr Engle has discussed this morning There is the old, old question of whether a man lives longer if he remains fertile. In rats we have found that sterilization by feeding diets with low levels of vitamin E has no significant influence on their life span. Recently, in our laboratory, we have had the first rat that could reproduce or rear a litter of young at the age of twenty-five months, which in human life would be comparable to perhaps seventy years or so.

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Fremont-Smith: Is this in the main artery?

Engle Yes, in the ovarian artery

Fremont-Smith Are you implying that for a while the artery had been actually completely obstructed before it was recanalized?

Engle: One gets that impression. I haven't done systematic work, and I am bringing it up because I want Dr. Steele and Dr. Oliver to make some comment. The problem has not been studied by anybody. The question is this: Is this premenopausal ovary, with limited steroid hormone production a result of this vascular pathology, or is it the cause of vascular damage? Does the lesion cut down the oxygenation of the tissue and limit the transmission of metabolites which in itself results in a more rapid loss of eggs, a slower development of follicles, and a general fibrous involution of the ovary? On this point we have no information, and I think that we do need some facts in order to look at the difference in the reproductive span of the two sexes. Why can a man be reproductively potent at eighty-five, and a woman stop at forty-five or so?

Cowdry: How much have you seen of these lesions?

Engle: They are very common; just in the daily run of the mill in a gynecological pathology laboratory.

Cowdry: A really occlusive lesion of the artery?

Engle: Total occlusive lesions, with subsequent canalization, are not common, the type of lesion in which the intima is gone, and there is a fibrous plaque, destroying the intima, and a great deal of fibrosis in the media, is a very common thing, extending all the way from the ovarian artery to the various radials up in the arterioles.

Fremont-Smith: How much of a cyclic change is there in the ovarian artery? I was under the impression that it, along with the uterine arteries, went through a fairly definite cycle with arteriosclerotic changes in it.

Engle: Such observations have been made in the rabbit but I don't know of any such observations in the human.

Oliver: I think that by and large, one notices in the wall of the uterus, the parametrium, and in the ovary, a sort of correlation of sclerotic lesions.

Engle: That's right They are not exclusively in the ovary. They occur in the myometrium and the parametrium.

Oliver: But the sclerosis that one finds in these organs is very real as compared to what one sees in testes I have never had any particular interest in this problem, but it occurs to me from my experience in looking at autopsy material that practically every old ovary has an extreme sclerosis of its vessels, and that is not true of every old testicle.

Engle: The damages in the vessels of the testis are not comparable in frequency and certainly not related to age as are those of the ovary If you are asking why the human female ceases reproductive life and begins involution earlier than the male, I think attention should be

focussed on the steroid balance, cholesterol metabolism, and the other [illegible]

[illegible] May I call your at-[illegible]ity years ago on the formation of hyalin in the ovaries (3)? In the earlier literature the hyalin masses in the ovary were considered under two headings: either as the result of follicular atresia or of hyalinization of occluded vessels In our study, however, we could show that a considerable amount of that hyalin originated in the stroma of the ovary entirely independently from vascular elements Through coalescence, garland-shaped hyalin masses are produced which are no longer distinguishable from the vascular hyalin or the hyalin masses derived from follicular atresia. As an end result, if all three components appear together, as they very often do, the hyalinization of the ovary is so extensive that hardly any parenchyma is left unaltered. Hyalinization of such a degree obviously has significance from the viewpoint of steroid production. At the time our study was made not much was known about steroid hormones nor did we study the presence of lipids in the cells. We did find, however, a rather interesting correlation; namely, that whenever the process of hyalinization was advanced beyond the average, one or several fibromatous tumors of the uterus were present; on the other hand, one could say that fibroma of the uterus occurs but seldom in the absence of extensive hyalinization of ovarian stroma.

Engle Yes, I remember that paper of yours, Dr Goldzieher I think we would all agree that these factors here are all interrelated, but I think it is fundamental to our concept—the difference in cessation of sexual and reproductive life

The other thing that I want to re-emphasize, because it is a problem for the cardiovascular people, is, why do we have this type of lesion associated with the reproductive organs in woman without evidence of much correlation between this type of vascular pathology and general systemic vascular pathology? I think that is important.

Oliver Of course, in both the ovary and the uterus you have destructive processes occurring all the time in cycles and you don't have anything like that happening in the testis.

Engle No, that is right.

Oliver That might explain the damage in the vessels of the ovary and uterus

Stern Isn't that a pretty general problem, this whole question of regional distribution of arterial changes? There are types of people, you might say constitutional types if that expression were not so vague, who have atheromatosis of the small arteries, and the large arteries are normal, and vice versa. In some cases, the cerebral, in other cases

the abdominal arteries are free of changes. I think this is just one aspect of the same problem.

Engle: That is right, except that this is a constant phenomenon in most women, isn't it? And various other types of atheromatous lesions are inconstant, that is, inconstant in relation to age or organ affected. That is why I think the ovary is very important and really deserves more attention.

Cowdry: We have made some experiments and observations on monkeys.

Engle: I have examined histologically the reproductive organs of a great number of adult monkeys. It may be important in evolution, but in all animals that we know, reproductive performance will continue throughout life. Beginning shortly after puberty, most animals will begin reproductive performance, which continues to the end of their life span. Woman does not do that.

For that reason, I was interested in the monkey. In our monkey colony at Puerto Rico, where we had free-ranging animals I was able to take a few of them at what I knew to be old age and examine them These old grandmas were reproducing, slowly, it is true, but when the reproductive system was examined, they still had lots of active eggs in their ovaries, and only a limited number had any degree of vascular pathology. But the monkey is a rather remarkable animal because one just doesn't see vascular pathology in the normal, free-roaming animals. Apparently the monkey doesn't have anything worrying him and he eats the right things.

From the standpoint of evolutionary change, how does it happen that all the lower forms have this reproductive facility and capacity, whereas in the human, intercurrent developments occur so there is an exhausted ovary with a damaged blood supply, with complete absence of specific sex hormones in the female In contrast, in the human male there is a longer period of reproductive life, slowly declining but highly effective hormonally. Why should the female experience a greater life span than the male?

Cowdry: I should think it would be remarkable if an occlusive change occurred in an artery only at the human level. Many of the differences between the sexes may not be easily related to life span.

Engle: That is quite true.

Cowdry: For instance, there are many other sex differences such as the difference in the hemoglobin content, the difference in the number of leucocytes, the difference in blood pressure, and a great many others. I don't know how much this tendency to deliver an insufficient supply of blood to the ovaries is an essential factor in bringing about differences in longevity between males and females.

Engle: I have not been trying to sell a bill of goods. I have been pointing out an area in which we have almost no systematic information, and I still think it is important to the problem.

Horvath: What about the ovaries of women who have been hysterectomized? Do they show the same pattern?

Engle: The ovary that has been left in after the uterus has been removed? Well, I can't recall when I have seen one of them. The usual procedure, if a woman is over forty years old, is bleeding, and has fibroids, is to remove all the reproductive organs

Horvath: I was just thinking that, after all, in the early days they didn't remove the ovaries and therefore there should be a fair number of people coming up for examination now, just as the result of natural causes

Engle: Yes, but I haven't seen them.

Horvath. Such ovaries should be available somewhere

Engle: The general pathologists are more apt to get those at autopsy, but I don't see them from the surgical side

Fremont-Smith: What about the ovary of the 20-year-old or 21-year-old woman in the cycle? Do the vessels go through changes? I think I asked this question before and it seems to me I got only a partial answer Are there arteriosclerotic changes at any time in the cycle of the ovarian artery of a woman in full sexual life?

Engle: That, I would not know, for the same reason, that I don't see that material

Fremont-Smith: Well, I wonder if anybody does know The implication was that there were very marked changes a little later on in life I thought this was something that had been brought out sometime before and that the occlusive changes that took place in the uterine arteries were paralleled by the ovarian artery—and this was a human observation

Engle: Oh, it has been described. People have commented on it before But, by and large, it has been commented on by the pathologist, who is not interested in steroid hormones, and I am pointing out now that with the new techniques in cytochemistry and endocrine analysis, we now have the tools whereby we can pick up this problem. I am not saying it is a new problem I think everybody has recognized it for years But I believe it is a significant problem and I think, with modern techniques, we could pick it up again and re-examine it and it might have something to do with the whole general process of aging.

Hamilton: I wonder if the sex differences in viability, in terms of longevity and life span, are not really due more to the male side than the female side of this picture? At least, it is my thesis that some of the lesser life span of the male arises from his higher rate of metabol-

ism, and that that is the particular disadvantageous factor rather than the female secretions *per se*.

Engle: I would like to ask Dr. Levin if he would say some things in relation to the possibility of shifts in metabolism of these steroids and any influences that they might have in the general aging process Nobody has mentioned here, Dr. Levin, the somatic actions of the steroids

Levin: I think my ideas are along similar lines as those I inferred Dr. Hamilton to have, if I interpreted his previous remarks correctly. I think if Dr. Hamilton had included his 17-ketosteroid data derived from men younger than age twenty, the first part of his curve would have increased rather markedly between the ages of 10 and 20 years. Is that right?

Hamilton: Well, yes.

Levin: A rather rapid increase in 17-ketosteroid excretion beginning at about puberty.

Hamilton: More of a straight line.

Levin: And after the peak is reached a few years after puberty, this more or less regular descent coincident with the phenomenon we call "aging." It is an accepted fact that in the male a large proportion of the urinary 17-ketosteroid is derived from testicular androgen In consequence, we can regard Dr. Hamilton's curve as more or less indicating the relative rates of androgen production throughout the life span. Another fairly well-demonstrated fact is that androgen, particularly testosterone and its derivatives, exercises a so-called protein anabolic effect, that is to say, it favors deposition and, presumably, synthesis of body protein. If we consider some of the matters discussed here today, including growth of axillary hair, the larger stature and greater muscular mass of the male as compared to the female, some of the things Dr. Cowdry mentioned such as replacement of cells, and so on . . . all of these are in one sense protein deposition and therefore in part an expression of circulating androgen concentration.

In the male we have a circumstance where protein deposition and replacement is occurring in a milieu containing higher concentrations of androgen than in the female. It may be—and here it becomes really speculative—that as the male grows older and the androgen concentration falls off, this results in a lesser rate of protein deposition or replacement and, in consequence, the gradual onset of a degenerative process at a rate somewhat greater than in the female who has never been exposed to such a high androgen concentration and whose protein replacement requirements are never quite as high as in the male Possibly this may be related to the faster aging and shorter life span of the male

Another hormonal factor to be considered in this connection is that fraction of the 17-ketosteroids, in the male and in the female, which is derived from adrenocortical hormones. These hormones are said to have a protein catabolic effect and are thought to be secreted in approximately equal quantities by the adrenals of the two sexes. So in the male you have a situation where the two types of steroids are metabolically opposed to each other. When the anabolic component, coming from the testes, drops out, the poor male is left with only the catabolic portion which would, in such a system, actually tend to hasten his degenerative processes and demise. In the female, on the other hand, there is no testosterone to oppose the action of the catabolic adrenocortical hormone and therefore one would have to postulate either some other counteracting hormone which does not decrease with early age as in the male or, as an alternative, that the female anabolic processes are inherently pitched at a slightly higher level in order to counteract the catabolic adrenal effects.

Certainly the degeneration of cells implies an *increased* catabolism or a *decreased* anabolism of cellular constituents such as proteins. And certainly as aging progresses, particularly in advanced ages, there is wastage and net loss of protein. Whether or not hormones are tied in with this as a primary agent is anybody's guess.

Shock: In considering data on urinary excretion of 17-ketosteroids, how can one separate the possible contribution of the adrenal from the gonads? Examination of the scatter plots of urinary 17-ketosteroids against age, published by Hamburger (4) or Kowalewski (12) indicates that at ages below 35 to 40 years, the values obtained in males are on the average *significantly* higher than those on females, whereas beyond the age of forty the sex difference disappears. Would not this indicate that at the higher ages 17-ketosteroid excretion is more a reflection of adrenal than of gonadal activity?

Hamilton: Well, I think we are all more or less biased when there are no facts and we have just hunches. I think the answer to that at present is only on the basis of guesses. The usual guess is what Dr. Levin has said, that the adrenal contributes a large amount in the female. I personally don't subscribe to that at all. I think the evidence is circumstantial on that matter. For example, if one uses axillary hair, which is a measurement I am more sure of than ketosteroids, and takes out the ovaries, the axillary hair almost stops growing, and if one takes out the testes, the axillary hair also stops growing. Any adrenal contribution to the axillary hair growth is at least minor.

If I may follow another train of thought in which I am more interested at the moment, I wonder if the group would care to comment on this. Is the lesser life span of males, in your opinion, related to higher

rates of metabolism observed in males? I have hunted all over the literature for length-of-life data on castrates and have never found it We have over a thousand animals now of different species, normals of each sex, and castrates, and are trying to answer that question. The only data I know of are on a colony of eunuchs and noncastrate men living in the same institution, where the eunuchs are longer lived and live almost as long as the females in that institution. This is the only evidence on this matter that I know of.

However, there is circumstantial evidence that with castration, all of the basal autonomic functions that we have been able to measure, decrease.

We had originally worked on only a few men before and after castration. We now have data for about twenty-five such individuals. These are especially good data in that the same man serves as his own control before and after castration. Decreases were observed in pulse rate, hemoglobin levels, quantity of erythrocytes per unit volume of blood, BMR, total respiratory volumes—anything we could think of as a basal function showed a decrease after castration If one is fatalistic and assumes that we have only a certain ability, genetically established, to synthesize protein, if you wish, or a certain amount of living to expend, then life at higher rates of metabolism would bring about a more rapid expenditure of this potential. I am not sure that we are dealing with higher rates of metabolism. It might be total expenditure. In some members of the insect world, certainly one can take the number of heart beats times the length of life and come out with a constant. I think that has been done by various people

Heilbrunn: I don't think so

Hamilton: Would you care to take over, Dr Heilbrunn?

Heilbrunn: I don't think there are enough data to prove that for insects

Hamilton: The data I was thinking of were specifically those for Daphnia by MacArthur and Baillie (16, 17) and for flies by Loeb and Northrup (15).

Heilbrunn: On the rate of metabolism?

Hamilton: No, on the heart rate and CO_2 production in the case of the Daphnia

Heilbrunn: The length of life in insects varies from a few hours to a dozen years, and it would be very hard, I think, to establish a constant number of heart beats for an insect, for varying life span.

McCay: When you retard rats, you have no sex difference at all; that is, the life span of retarded rats is the same for both sexes. But when one thinks of the relation of basal metabolism to age, I wonder if there aren't species differences. As far as I know, there is no good evidence

in the literature for a decline in basal metabolism with age in any experimental animal other than man, that is, dog data have been negative and rat data have been negative

Shock: I believe Kunde and Nordlund (13) found no change in basal metabolism in dogs kept under laboratory conditions for 2 to 12 years. However, Davis (2) has reported a fall in basal metabolism in the rat between the ages of 6 and 30 months that was very similar to that observed in humans between the ages of 20 and 65 years

Lerm: I think it has been rather definitely shown that the male rat has a higher spontaneous activity than the female Certainly if castrated, the spontaneous activity of the male decreases and if he is then given androgen in order to replace the endocrine secretion of the testes his spontaneous activity increases again I believe this was shown by Hoskins and perhaps by others also

Shock Slonaker (19, 20) has shown that castration diminished spontaneous activity in rats

Stern Did the basal metabolism increase again with the administration of androgens?

Lerm. I don't know whether [illegible] castrate male rat causes an incr[illegible] consumption I am inclined to s[illegible]

Fremont-Smith. It should be possible to determine the relationship between energy output and longevity *in rats* One could compare the longevity of rats that were put in a cage and run for long times with similar rats of the same sex which did not run. It should mean, if the implication is correct, that the rats that ran and therefore used up much more energy and had a higher metabolism through a much larger portion of their daily life span would have a shorter life span Now, is there any evidence on that?

McCay Yes, we did that experiment The rats that run tend to keep thinner and live longer

Fremont-Smith. That is what I thought

McCay If you don't start them when bronchiectasis is advanced. If you start running a rat then, you just kill him off Between breeds of dogs, the life span of the smaller breeds, the terrier, for example, is much greater than that of, say, the St Bernards and the Danes The terrier group is far more active than large breeds

Hamilton. There is also evidence, returning again to the Daphnia and to the fruit flies studied by Loeb and Northrup that the sex difference in viability and longevity disappeared when the higher rates of metabolism in the males were reduced In the Daphnia this reduction was brought about by changing the water bath temperature. In other words, both sexes lived longer in a cooler water bath (at 8° C.),

and they both lived almost the same length of life; whereas when they lived at, say, 18° C., there was a longer life span of the females. When the water temperature was further increased to 28° C., that is they lived at still higher rates of metabolism, the sex difference in longevity was still further accentuated. Relatively speaking, the females far outlived the males, although both sexes died at an early age.

Stern: To come back to the lipid cells, in the central nervous system in man, the most puzzling fact is that when you take the accumulation of lipids as one of various morphological signs of aging, there is a tremendous local difference, and it is quite constant. For example, the Purkinje cells, even in a 95-year-old individual, show hardly any lipids at all, and the cells, let's say, of the column of Clarke or of the inferior olivary nucleus begin to show lipoids in the early twenties and increasingly with age. In the central nervous system, there is this whole problem of local aging, which is something which has actually never been investigated systematically.

Heilbrunn: The histological detection of lipids would actually only indicate the free lipids present, wouldn't it?

Stern: Yes; actually, lipochrome, which is more or less identical with brown pigment, I think. But even so, if you get just the so-called brown pigment with these methods, again, it is biologically a most intriguing question, as to why, let us say, the cells of the column of Clarke or certain cells of the magnocellular nucleus in the dorsal part of the medulla, the so-called nucleus of Monakow, show a tremendous amount of lipoid very early, and other cells, for instance, the cells of Purkinje, even in senile subjects or people with senile psychoses, hardly show any lipoids at all.

Cowdry: Your idea is that endocrine products reach the cells almost equally and that differences in rates of aging are not correlated with the differences in blood supply, is that right?

Stern: These so-called senile changes are remarkably independent of vascular changes; in fact, it is almost as if there were a paradoxical relationship. Something which is often neglected in the textbooks of psychiatry, I notice, is the fact that senility and arteriosclerotic brain disease are two different entities, and so it is that senile brains, usually brains of people who died from senile dementia, show remarkably well-preserved, juvenile types of arteries, and the other way around You have brains with arteriosclerosis, and the so-called senile changes are not at all in proportion to the arteriosclerosis. That seems to exclude to a certain extent the solution you have just offered to this problem, that it may be just a difference of blood supply which is responsible for this whole aging difference.

Horvath: We haven't been able to find any difference in the blood flow to the brain with age; that is, just by chance analysis of people who come in to have blood flow studies done There is no difference. The flow is still within the range of 50 to 60 cc. per 100 grams of brain per minute.

Goldzieher: If I understand Dr. Cowdry correctly, he meant that because the changes are localized they could not be referable to the effects of hormones brought there by the blood. I could not quite agree with that view; though at a given blood hormone level the same amount of hormone reaches every tissue and cell, the responsiveness of the target organs remains variable. Thus a certain hormone level may be maximal for one tissue and not adequate for another. Taking this variability of target organ response into consideration, the observation would not rule out the effect of hormones or their absence on the production of changes.

Cowdry: I am glad you have raised that point, because the areas in which Dr Stern described these nerve cells, one showing a heavy amount of lipochrome and the other one not, were right *next to each* other so that one would expect them to receive approximately the same supply of hormone, unless there is a sharp difference in blood supply for which there does not seem to be any evidence I think that you have raised an extremely important point; that is, the responsiveness of the target organ This is a conspicuous feature of aging, particularly in the reproductive system

Frank That change in responsiveness becomes very relevant, if the gonads are no longer selectively absorbing the hormones in the blood, does that alter the hormone balance? Then what is happening to the whole balance of the body? There have been many observations during the menstrual cycle in the woman between fifteen and forty-five, showing various fluctuations; basal rectal temperature, BMR, water balance, weight, and so on As I understand it, these fluctuations diminish considerably after involution, so that you have an organism whose amplitude of fluctuation has been considerably diminished after involution How does that fit into the pattern of prolonged life span? It is true, isn't it, that those fluctuations diminish?

Stern. Yes, that is a fact

Hamilton May I throw a question in here? The paradox that has been bothering us for quite a while now is this· If there is, as there well seems to be from several lines of evidence, a decrease with age in the amount of stimulating steroid substances, it is quite a paradox that many of the conditions produced by these steroids have an increased incidence with age I will cite just two conditions One is baldness, which we know is a male hormone-incited condition (6).

Another one is prostatic cancer, which has some apparent dependence on the steroids (9, 10, 11).

I think there are many of these androgen-influenced conditions that increase in incidence with age, while the titers of the stimulating substances are apparently decreasing. That is, I think, a very important paradox. We made the tentative assumption that the role of the androgens in things such as baldness was that of simply setting the stage; that is, one had to have threshold amounts but, beyond that, the controlling features were not greater quantities of androgens but were changes in the responsive tissues, as influenced by age, intercurrent events or other factors.

Engle: I don't think there is any paradox there at all. You are just dealing with a difference in the life history. If you will forgive me, I will not make any comment about baldness, because I don't know anything about that, but since you bring up the problem of cancer in relation to steroids, the relationship of ovarian steroids to endometrial cancer and testicular steroids to prostatic cancer is just too close to be ignored. But in the natural history of the disease, we know there is a declining incidence of androgenic substances and an increase in the incidence of prostatic cancer. But prostatic cancer is a long, slow developing disease I have seen microscopic evidence in autopsy cases in relatively young men, where you know it would not be clinically diagnosed for another twenty or thirty years. I think the same thing is true of endometrial cancer. It is, of course, an aphorism among gynecologists that the woman with a bloody menopause has a frequency of carcinoma of the corpus uteri of about eight times that of the uneventful menopause. Well, that bloody menopause indicates that you still have an ovarian function that is struggling It does not drop out of the picture at once There is an increase in the number of atretic follicles, and therefore an increase in steroid production And there, again, the cancer-favoring process operates over a very long period of years.

It is true, I think, that there is a certain biological substrate that is more or less susceptible to the development of carcinoma in these specific target organs that are responsive to steroid stimulation For instance, the correlation between breast cancer in women and subsequently, ten or twenty years later, the development of a carcinoma of the uterus, I think, is just too high to be regarded as being happenstance. I think there is a very definite relationship between these two organs which are target organs for the estrogenic steroids and eventually develop cancers.

Cowdry: As I understand it, the figures show that if you have one

primary cancer, the chances of having a secondary primary cancer are six times as great. Isn't that the case?

Engle: Something like that.

Cowdry: And doesn't that apply to other cancers besides those you have mentioned?

Engle. The only other association I know is what Moore pointed out. He was studying prostatic cancer, and the most frequent primary cancer occurring in relation to prostatic carcinoma was in the thyroid. In women, I am almost certain that the highest correlation is between breast and endometrium.

Cowdry. Yes, but if you are really considering frequency from the statistical point of view, wouldn't you agree that the tendency to production of squamous cell carcinoma of the epidermis is really very high indeed and ignored because such tumors are always cured or nearly always cured?

Oliver. Yes, I think it is.

Cowdry. I think that the remark about protein formation under the influence of testosterone is very important. This should be further discussed. There have been no remarks as yet about the turnover of labeled substances in various tissues. Have we any accurate data for consideration? As I recall, the turnover of labeled calcium is very considerably reduced in epidermal cancer compared with that in normal epidermis. We have this kind of evidence to consider, as well as differences in the total amounts of calcium and other materials. The increase in calcium with age in the medial layer of arteries of males is greater than it is in females. Isn't that so, Dr. Steele?

Steele. Yes, I think it is.

Cowdry. The decrease of calcium with advancing years in the bones of males is, I believe, greater than that in females.

McCay. It is true in the rat, but I don't know about the human.

Engle. What happens to phosphorus in those instances? The calcium shifts, does the phosphorus shift inversely with it?

Cowdry. I don't know.

Lerin. Are you now referring to changes in calcium content of bone?

Cowdry. We spoke first about epidermis and then shifted to bone.

McCay. I was talking about the calcium in the bone, that is, the density of rat bone. When animals die of old age, the female dies with denser bone, in spite of the fact that she lives much longer.

Cowdry. That supports what I said, doesn't it? — that in the male, the lack of density (reduction in calcium) is more marked in aged males than it is in females.

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Cowdry That supports what I said, doesn't it? — that in the male, the lack of density (reduction in calcium) is more marked in aged males than it is in females

McCay. That's right

Levin: This is very interesting for it is in perfect correlation with the demonstration by Gardner of Yale that estrogen administration to mice for long periods of time leads to spectacular deposition of bone. In some of his animals almost the entire marrow cavity was filled with solid bone. Here again you have an endocrinological influence.

Engle: That, again, is only in the mouse and chicken.

Levin: That is right. I'm not aware of any such demonstration in humans but then again, the comparable experiments probably haven't been done.

Goldzieher: Clinical experience in humans which, of course, does not represent well-controlled experiments, shows that male patients with osteoporosis do very well on testosterone administration alone. Female patients with osteoporosis given estrogens do improve but not nearly as rapidly and completely as if testosterone is given simultaneously or alone without estrogen. In other words, in the aging human, irrespective of sex, testosterone is more potent in improving the calcium balance of the osseous system than estrogen.

Levin: I think that in this connection we must consider the formation of bone in its entirety Bone consists of two distinct portions, a protein matrix and the minerals, calcium phosphates, carbonates, and so on, which are deposited on or in the protein matrix. I don't believe real bone formation occurs without the prior formation of a suitable matrix and to get this protein deposited I should imagine a protein anabolic substance would be of considerable benefit It is probably for this reason that testosterone, and also growth hormone, are of value.

Goldzieher: That sounds very good and is indubitably correct biochemically, but *it is hardly acceptable from the viewpoint of the pathologist.* In the osteoporotic aged bone, there is still some of the protein matrix left. It shows evidence of halisteresis, i.e , elution of the calcium salts. Decalcified protein matrix may be present fairly extensively in senile bone tissue.

Cowdry: A change in the composition of protein matrix may occur.

Goldzieher: That is possible

Engle: I think that may be the difference, because I am quite sure that Dr. Levin's point is correct. If you are dealing, say, with retarded growth in rats, or dwarfism, the primary function of the use of testosterone is to get an adequate protein matrix laid down first, and then the calcium phosphates and carbonates are deposited. However, in aging you are dealing with an involution of the bone and you have no way of knowing what the change in the protein matrix substrate is

Goldzieher: That is right.

Fremont-Smith: May I make a cross reference to our conferences on metabolic interrelations (18)? I remember that Albright started out

with that premise, that it was first necessary to lay down a protein matrix into which calcium salts could be deposited and that, therefore, by use of androgens, one would be able to facilitate bone healing. Now, I am under the impression that that never could be demonstrated to work in experimental bone healing. I believe there is some evidence suggesting that calcification takes place simultaneously with matrix formation, rather than in a two-step process—there, again, coming to a quantitative interrelation between quantity of matrix and quantity of calcification

Cowdry: Wouldn't it have to do with calcium binding by protein? Of course, the unbound calcium is going to be deposited unless—

Fremont-Smith: The whole nature of what bone is chemically, and even the nature of the calcium salts and the state of calcium in the blood, are not clearly understood, as far as I can make out (18).

Cowdry I was thinking, of course, in this connection about what I hope to say tomorrow morning, as to the relationship of the proteins and the amino acids and the fibers to the accumulation of calcium.

Shock Is there any evidence that in older animals, when you stimulate calcium retention, all of the calcium goes to the bone and not to other soft tissues where you don't want it?

McCay: We have tried to get at that for fifteen or twenty years and never had any evidence of soft tissue calcification related to diet.

Shock: But, neither the dog nor the rat is subject to the deposition of calcium in soft tissues

McCay The rat is very much subject to it, I should say We see heavily calcified soft tissues, especially the kidneys, in our rats. We pick them up under the X-ray all the time We have never had a correlation between holding an animal in balance and more calcification in some of the soft tissues as a result of doing so

Shock: What I am getting at is, is it possible that one might do more harm than good to an aging animal by increasing its calcium retention?

McCay We never had any evidence that when we hold rats in calcium balance by giving them more assimilable calcium, they get more calcification, say, in the kidney, where the rat is very sensitive to calcification

Shock: What about the patients who are treated with hormones for osteoporosis? Is there any evidence that the vascular system suffers as a result?

Goldzieher: We have no such evidence and at least so far, no autopsy findings are available At this point I would like to come back to the question of calcium deposits in the protein matrix which is either a newly formed or an old one; the latter persisting after some process of decalcification The amount of calcium salts which can go into that

matrix is highly variable; in other words, a given protein matrix may show incomplete calcification—calcification comparable to that of ordinary bones, or it may turn into extremely sclerotic bone tissue with a much higher than normal calcium content. Therefore it would seem that the protein matrix is not the only determining factor which regulates the deposition of calcium salts but that other forces must be also operative, be they local (pH) or humoral (steroid hormones).

Cowdry: You will admit that when you look at the protein matrix, you can't definitely say whether it is changed or unchanged?

Goldzieher: No, one cannot.

Cowdry: It may very well be that there are differences in the protein matrix that we know nothing of.

Goldzieher: That is right.

Fremont-Smith: Causing a difference in the matrix itself; in other words, a matrix which accepts and doesn't accept calcium

Cowdry: They could be very different.

Levin: As far as the two-stage reaction in calcification you mentioned is concerned, I did not mean to imply that it is of necessity a one-two reaction. As I recall, in the flat bones of the fetal head one sees not only early calcified bone but also surrounding it a noncalcified substance which the histologists call osteoid This is explained as being the protein matrix and that the calcification process is advancing into it as the bone develops and grows I think one has to admit that at least in this case the formation of protein matrix precedes the calcification process.

Fremont-Smith: I think you are quite right on that I think it counteracts the statement that it is always simultaneous, because, obviously, in that case it is not.

McCay: I was thinking of the sex differences that Albright points out in his book on the parathyroids (1). His X-ray photographs of patients with postmenopausal osteoporosis show characteristic changes in the bone, but no analogous bone changes are reported in aged males Is there nothing in the male that compares with that menopausal calcium loss?

Engle: No, I don't know of any other phenomenon The frequency of clinical osteoporosis is, of course, very great in the female

Hamilton: The radiologists report bone changes after castration in the male

Steele: But isn't it interesting that this protein or calcium-retaining action of testosterone does not seem to be linked with androgenic stimulation? We have been using 17-methyl-androstanediol that appears to have none of the male hormone effects, and yet we can get retention of protein. I think the work of John E. Howard at Johns

Hopkins Medical School showed that if you did not get the patient in protein balance, you could not make him store calcium. Dr. Cowdry is, of course, perfectly right. There might be something else wrong. Calcium is not always retained when you have protein balance, but retention of calcium and repair of the bone do not usually occur without a positive nitrogen balance.

Fremont-Smith: Personally, I think starvation couldn't heal a bone There must be a lot of clinical evidence on that

Cowdry: Does 17-methyl-androstanediol act like a metabolite?

Steele: It seems to have the same anabolic action as testosterone

Engle: It acts like testosterone in its anabolic action in relation to protein, weight, growth, new protein deposition, and calcium retention, but it does not have the genital activities of testosterone It seems to be relatively inert in activating the genital system

Goldzieher: Yes, I've had the same experience.

Engle: We are using it quite extensively now for the purpose of utilizing a substance which will act like testosterone in the general somatic metabolism but have no action on the genital organs

Hamilton: I wonder if we don't have to consider more than general body-wide actions here? For example, many of these anabolic actions of androgens, particularly testosterone, can occur locally One can apply some of these androgens to discrete areas and get a growth stimulation in those areas without any general effect over the entire body I don't know that it applies to the calcium problem, but it certainly does to certain other tissues such as the skin

Fremont-Smith: In line with that, does my question come in about the healing of a fracture in a starving individual or a starving animal? Is it possible that there could be a redistribution of protein so that it would be available to make matrix? There must be some clinical evidence on that, where people have been injured, have had fractures, and have not had food Is there any evidence that they don't heal? Can you answer that, Dr Steele?

Steele: I don't think I can, except that they knew that bones with fractures did heal in the Japanese prison camps, and those people were presumably not in nitrogen balance But there isn't any direct evidence on it because as starvation progresses—if they are just getting a little bit of protein, nitrogen excretion diminishes so that you can't be perfectly certain whether they were in nitrogen balance or not.

Levin: Dr Fremont-Smith, in connection with the redistribution of protein during protein deficiency as in malnutrition, I have always felt that the organism is rather efficient in a homeostatic sense. For example, even in fasting the body will make use of its own proteins from muscle and other sources to synthesize and maintain a proper level of

a vitally necessary protein such as serum albumin. Of course, this mechanism will finally break down after long-continued protein deficiency but for a while, at least, here is an example of protein redistribution which obviously maintains a vital substance at the expense of others which aren't quite so necessary. I would think that a similar protein redistribution, making possible bone or wound healing, would also be within reason.

If I may, I would like to go back to the paradox which Dr. Hamilton raised. If we superimpose on Dr. Hamilton's curve another to indicate adrenocortical secretion during the life span, and if we then calculate the ratios between the androgen which is protein anabolic and the adrenocortical hormone which is protein catabolic, you can readily see that we get a constantly changing ratio between these two opposing types of hormone activity. This, in turn, may have a real effect on the metabolic activity of, for example, the hair follicle which is laying down protein in the form of hair. The changing hormonal ratio thus may of itself be an important factor in causing changing rate of hair growth or other such local actions I think finally we must come down to the physiology of the cell itself, about which Dr. Heilbrunn was speaking during the intermission, and to the effect of these metabolic hormones on the intracellular processes because ultimately we are dealing with the collective metabolism of individual cells.

Heilbrunn: My name has been mentioned, so may I say one thing? I found this discussion extremely interesting, and it is very pertinent, of course, to the aging of the testis and the ovary But in my opinion, for the general problem of aging, the ovary and the testis are not in themselves of primary importance because, as has been pointed out here, animals without ovaries and animals without testes have no very different life span from normal animals It was shown years ago that castrated dogs had no very different life span from those with very active interstitial cells in the testis; so that although the problem is of very genuine interest in regard to the aging of the testis and ovary, the use of androgens or estrogens does not appear to be of primary importance in the aging problem over-all—or at least so it seems to me I would be glad to be corrected

Steele: Does anyone know what the difference between males and females would be if mortality were reckoned without including coronary thrombosis. Do you know, Dr Simms?

Simms: I haven't made any comparisons except on chronic nephritis and tuberculosis. In both cases the women have a higher death rate below middle age and the men after middle age

Fremont-Smith: That would not be very hard to calculate, though, would it?

Simms: No, it would be very easy.

Heilbrunn: There are other factors, though. A man goes to work and gets into an industrial accident or is hit on the street.

Steele: You could pick out a few things like that. It would be interesting to know what remaining differences existed, and the specific things that made the difference.

Hamilton: I would challenge whether coronary thrombosis should be excluded What right have we to assume that this condition, which has a higher incidence in males than in females at early age of life, [illegible] rce of your

[illegible] (8).

Goldzieher: With respect to the seemingly paradoxical effect of steroids, I have to bring up again the importance of the responsiveness of the target organ which explains why we may observe hormonal effects at even lower than normal levels of hormone production and circulation As a specific example, I give you the hypogonad male. The hypogonad male, at the time of puberty, is almost invariably affected with a great deal of acne—far more than the young adolescent; this acne does not subside spontaneously even past the age of adolescence whereas the disease is self-limiting in the ordinary adolescent. Yet steroid hormone production is much higher in the latter than in the hypogonad It is known, moreover, that acne is produced by the direct effect of the male sex hormone on the sebaceous glands of the skin Nevertheless, the hypogonad, in spite of his low androgen production develops acne and carries it well into adult life This paradox must mean that the response of the skin and its sebaceous glands to steroid stimulation is different in different individuals one responds excessively to a lower level of stimulation whereas another does not react even to higher dosage

Hamilton: May I add a comment? We have had a chance now to study a great many people who were castrated before maturity and in not one of them has acne ever occurred; so there is a threshold level of testis secretion necessary for acne We have never seen it in any person who was deprived of testicular function during the time for sexual maturation

Goldzieher: What I said is true only in respect to the hypogonad, not the castrate I agree with you absolutely

Hamilton I was trying to clarify the issue. The term hypogonad, that you are referring to, does not mean absence of testis secretion because in its complete absence no acne develops. Moreover, castrates show very low values for sebum as collected from the skin in various surface areas.

Engle: Dr. McCay, I suppose you will want to be closing the session shortly, and I just want to make one statement on which I had really planned on getting a discussion started, something on the role of the thyroid, which hasn't been mentioned. There has been only casual, incidental mention of the adrenal cortex and the parathyroids here this morning, all of which are important in this problem of aging. If this morning session has been devoted more or less to the ovary and the testis, that is just too bad. It reflects my own interest rather than the problems of aging. That means we have to have another session some other time for those other important endocrine organs.

Fremont-Smith: Doesn't the discussion this morning illustrate very nicely the necessity for doing two things simultaneously, which we have in a sense been doing here? One is to speak of specific organ cells, and, for the other, to look at the Gestalt, the totality of the growing organisms, and that we cannot possibly understand any process and certainly not the process of aging unless we keep looking at the Gestalt within which these particular and more specifically interrelated processes take place.

Cowdry: As the philosopher says, if we understood everything about the little finger we would understand all about the whole body. These very remarkable regional differences that have been mentioned, together with the profound general differences, the differences in responsiveness as well as the differences in amounts of substances in the circulation, make a mixture of events extraordinarily difficult to analyze. But I still think the kind of data that will be most helpful are the rates of turnover of labeled materials in the several tissues of the body.

McCay: You see regional differences in radioactive work where the turnover of radioactive calcium is much greater in the vertebrae of old animals than it is in the long bones, such as the humerus or femur We see that constantly in our old rats and dogs, where one finds different rates of turnover in different parts of the skeletal system.

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CARDIOVASCULAR ASPECTS OF AGING

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I WOULD like to say that I am delighted to be here. It is the first of these meetings that I have attended, and Dr. Hoskins didn't give me very many instructions. Not knowing what was wanted, I really didn't prepare anything

Fremont-Smith: That is just what is wanted.

Steele: Good! It always seems to me a very hard thing to try to distinguish between whether something is a disease or a normal aging process, and I think every one of us has increasing difficulty in trying to separate the two in our minds. If in one particular organ or system there are changes that outrun the changes in the other organs, I suppose we tend to think of it as a disease, whereas if all of the organs tend to deteriorate at roughly the same speed, then we are more apt to think of it as a natural process moving with time. The longer you live, certainly the more chances you have of some "bug" hitting you, or almost any type of accident, and, perhaps, the longer you live, the more chances there are that some of your organs will wear out.

When it comes to cardiovascular disease in aging, I am afraid my mind is one-track I do not see how you can get around the fact that whether it is labeled "disease" or "aging," the common garden variety of arteriosclerosis is about 99-44/100 per cent of the problem Now, I am perfectly aware of Dr Lansing's beautiful work on the continued changes that occur in arterial walls—the increase in calcium content, the changes in the enzymes and amino acids which seem to change with time and which precede the development of arteriosclerosis (26, 27). There may well be something that happens to the arteries in preparation for the subsequent irregular development of arteriosclerosis, but at least as far as the health of older people is concerned, arteriosclerosis is the most important disease I have always thought that it was perhaps a pity that Dr Osler ever made or repeated the statement that "a man is as old as his arteries" It has intimidated a great many people from setting about the study of arteriosclerosis Thinking that way

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Well, these observations set me onto another trend, and that is something about which I would like to hear this group's opinion. I have a notion that, ma ... one has to study arteriosclerosis ... None of these people had str ... coronary occlusions or anything else I wonder if the disease can't really become stationary for a good period of time During that time the basic factors might not be evident.

Of course, one thinks of hypertension as one way of showing a relationship of wear and tear to the arteriosclerotic process. Where the greatest strain is, where the greatest pressures are, and when hypertension is present, there is usually more arteriosclerosis observed than otherwise Of course, at the present time, so much has gone into the study of lipids and the association of lipid disturbance with arteriosclerosis from the experimental approach, from Anitschkow (2) on down concerning the relationship between high cholesterol levels and the appearance of the lesions, that almost all the studies of arteriosclerosis today deal with some form of measuring or some way of studying lipid metabolism. Everybody knows of the extraordinarily good work Steiner and Kendall and their associates have done on dogs (40). The disease which they were able to produce in dogs by feeding cholesterol and the antithyroid drug, thiouracil, simultaneously was more nearly a facsimile of human arteriosclerosis than prior experimental attempts The objection raised to the use of rabbits who ... terol-phospholipid ... in either the dog ... occurred. Kendall developed the notion that this ratio was important and that as one approached the ratio found in man, one might develop arteriosclerosis spontaneously.

With that point of view in mind, we thought that perhaps, rather than just phopholipids in general, one fraction of the phospholipids might be more important than another. Lecithin makes up about 70 per cent, sphingomyelin 15 or 20 per cent ... cent of the total phospholip ... that some individuals do n ... all ... to hy ... from ... by d ... with cholesterol and phospholipid values all over the lot and barn, the ratios between the various phospholipid fractions themselves as opposed to the variability of the ratio of phospholipid to cholesterol did not change The ratio of

strongly suggested that the process was more or less part and parcel of a natural process and that no one could, therefore, do anything about it. Nevertheless, the evidence is quite clear that there are possibilities of influencing the development of arteriosclerosis without ever really understanding its complexity or origin.

It seems to me there are many ways of going about its study, first, from the point of view of racial characteristics and dietary habits of peoples. I have always been impressed with the sort of loose information that comes back from Okinawa and from China and from Malaya, that the various races have less arteriosclerosis than we, and yet, from the data as they come, it is hard to distinguish whether it is a hereditary affair or whether it is a difference in habits, chiefly eating habits. At Goldwater Memorial Hospital, we have had something like fifteen Chinese patients die. They ranged from fifty to seventy-five years, and I don't think there have been better examples of generalized arteriosclerosis by X-ray in any group of individuals than in the majority of those Chinese

It has occurred to me that if one could study the Chinese in China and the Chinese in America, and the Americans in China and the Americans in America, by the same methods, with comparable groups and comparable techniques of analysis, one couldn't help but come up with some answer as to whether differences in degree of arteriosclerosis were due to dietary (environmental) differences or were racial characteristics.

I have been trying to carry on a study of a small number of people over a long period of time, rather than to collect statistical data on many subjects. There is a group of fifty to sixty persons that we have followed over a period of nearly ten years We had thought that we might carefully document the kind of food they ate, the sort of people they were — whether they were vegetarians, of what extraction they were, or what their family eating habits were — and try to correlate these facts with the presence and further development of arteriosclerosis. It became obvious that the means by which you can document the presence or absence of arteriosclerosis in human beings are extraordinarily poor. You have to rely on some indirect sort of happening like a coronary occlusion or cerebral hemorrhage or on serial X-rays, picking up the addition of calcium in the aorta or in the vessels of the leg. What has really amazed me is that in these sixty people, the arteriosclerotic changes have really been more or less static. There wasn't enough change in degree of demonstrable arteriosclerosis during the whole period in these persons who varied from fifty-five to seventy years of age, to correlate with anything.

Shock: Hallock (16) has also published observations that lend support to the idea that changes in pulse wave velocity may occur in the absence of plaque deposition in the artery. As I recall the study, Hallock found a significant increase in the radial pulse wave velocity of young treated diabetics (mean age sixteen years) who had no gross evidence of arteriosclerosis. Beyerholm (3, 4) has also reported an increase in pulse wave velocity with age but found no differences between subjects with clinically detectable arteriosclerosis and those without.

Steele: I was interested in hypertension at the time, and I showed that the hypertensive's rapid transit could be ascribed simply to the increase in the diastolic tension of the artery rather than to any thickening or stiffening of the artery itself (39). Hypertensive arteries formed a normal curve if you corrected for increased diastolic pressure.

Shock: We have been very much interested in the elastic characteristics of blood vessels, and Dr. Landowne in our laboratory has set up an experiment for measuring the pulse wave velocity at different intra-arterial pressures The technique is simply to superimpose a mechanical impact on the normal pulse wave curve, so that you get a series of pips on the normally fluctuating pressure curve. He has been relating the conduction time to pressure for each individual. We feel that that may give us an index of the elastic characteristics of the vessels that would permit some estimates of changes with age

Steele: I guess I don't quite understand what the additional impact adds to the measurement

Shock. Well, you see, the Hallock technique really determines the pulse wave velocity at diastolic pressure. By applying impacts of known amplitude and frequency to the vessel in the upper arm superimposed on the normal changes in pressure in the artery and then measuring pressure changes through an intra-arterial needle in the same vessel at the wrist, we can estimate transmission time at different blood pressures in the same individual It is also possible that we can determine the effect of the nature of the vessel wall on the wave form induced in the blood vessel by the impactor.

Steele So that you can get the pressure time wave for various pressures in each particular artery?

Shock: For each individual

Steele Yes That sounds like a very profitable thing It is a cute trick

Shock: Of course, I [illegible]
in the large blood ve[illegible]
many old men with n[illegible] [illegible] in their large vessels, who

sphingomyelin to lecithin was so constant that it was not possible to draw any conclusions as to which fraction was more important. The proportion of lecithin to sphingomyelin was the same all the way up and down the scale. It looks very much as though determination of the total lipid phosphorus gives about as much information as fractionation of phospholipids.

I don't know how to go on from here. I would like to know what notions other people have for studying the cardiovascular system in aging, from the point of view of something other than arteriosclerosis, something like the things that Lansing and his group are doing. Dr. Engle suggested a really intriguing approach, and I would like to ask him what is known about the way in which the umbilical arteries disappear. What happens to them? What are the changes in the uterine *vessel and the ductus arteriosus? Are the changes which occur in them* in your opinion in any way similar to the degenerative changes that take place in the common garden variety of arteriosclerosis?

Engle: That is extraordinarily interesting. I must say honestly that I never thought of it before. Certainly, I don't know whether the intra-abdominal umbilical vessels thrombose at the time the cord is cut and eventually fibrose Who would have some interest in that problem? It should be examined.

Steele: There is something known about it, but my knowledge is limited to some textbook pictures. The pictures of degeneration of the uterine vessel are, to my way of thinking, different from the process we are talking about. They are more like the changes you see, perhaps, in the arterioles with hypertension For this reason I had not thought of it as a profitable way of trying to study arteriosclerotic disease But when you think of the rhythmic changes that you spoke of this morning in the ovary I wonder what kind of lesions a repetition of such a degenerative process might produce.

Shock: In these patients that you have followed, have you used pulse rate velocity determinations as an index of changes in the large vessels?

Steele: I have not made measurements of arterial pulse wave velocities since those early studies that were related to hypertension (39) rather than to arteriosclerosis. Hallock (15) made a beautiful study of it, and I suppose that might be one way of documenting the change in arteries beyond just simple clinical observations. The only trouble with it is that it measures over-all rigidity I think Hallock showed an increase in speed, that is to say, stiffening of the arterial wall in the absence of arteriosclerosis It is measuring something other than deposition of plaques, although plaque formation must contribute in some way to the stiffening of the wall

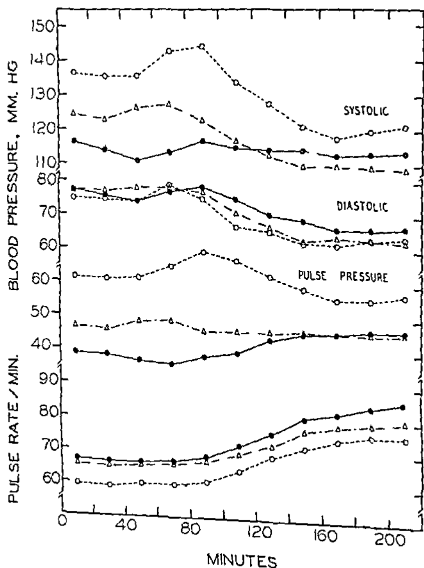


FIGURE 1 * Changes in pulse rate, systolic, diastolic, and pulse pressure during pyrogen reaction.

Fifty million killed typhoid organisms were injected intravenously at O time

o- - - - o Mean value for 34 subjects in O group (70-85 yrs)
Δ—·—Δ Mean value for 20 subjects in M group (50-69 yrs)
●——● Mean value for 20 subjects in Y group (20-49 yrs)

*Figures 1, 2 and ...
as a fac
self 30 .

are able to get as much blood through their kidneys as the average nonsclerotic forty-year-old (5). The important question, physiologically, seems to concern the status of the arterioles and small vessels. Perhaps investigations of changes in blood flow in other organs and the periphery, using plethysmographs, would give us important information about the functional status of the arterioles.

Steele: I am glad you brought out that point because it is amazing to me. We have done the same thing in the study of the peripheral blood flow that you have done with the kidney. Unless an artery happens to be blocked, and there is no pulse in the leg and it is cool, you can have an X-ray that suggests just what you have described—a lead pipe right down to the toe, and a blood flow and a vasodilator reaction that is quite normal. With vasodilators, you can get perfectly normal increases in blood flow through the foot with lead pipe arteries all the way down to the toe. This means, of course, that whatever sized arterioles are affected in hypertension, are not affected in arteriosclerosis

Shock: I would like to show some slides to illustrate the changes in renal blood flow that can be experimentally induced in older subjects This work was carried out in our laboratories in collaboration with Dr Roger K McDonald and Dr. David H Solomon (28). As you know, Dr Davies and I found a marked reduction in effective renal plasma flow with increasing age (5). The question was whether this reduction in flow could be accounted for by structural changes in the blood vessels in the aged, such as diminution in caliber and obliteration of pathways, or whether there were functional changes in the degree of vasoconstriction of the renal vessels. Furthermore, we wanted to find out whether the small blood vessels in the old kidney were still capable of responding to vasodilators. Therefore, in these experiments, effective renal plasma flow was measured in old and young subjects before and during the administration of a pyrogen. (50,000,000 killed typhoid organisms as 0 05 cc TAB vaccine were injected intravenously.) The 54 male subjects studied were divided into three age groups: young (Y) age range 20-49, mean 36 6, N = 20; middle (M) age range 50-69, mean 58.8, N = 20 and old (O) age range 70-84, mean 76 9, N = 14. Figure 1 shows the average changes in blood pressure and pulse rate for the three age groups You can see that the older subjects (dotted line with open circles) showed a rise in systolic blood pressure following the administration of pyrogen (at 0 time) whereas the young subjects (solid line and dots) showed no change The diastolic blood pressure fell in all age groups and the pulse rate increased

showed a significant increase in effective renal plasma flow. The absolute increase was not as great in the old subjects as in the young, but on a percentage basis the increments were of the same order of magnitude (Y = 71%, M = 86%, O = 91%).

This figure also shows the change is filtration fraction for the three age groups. You will note that at the beginning of the experiment, the old group (dotted line with open circles) had significantly higher filtration fractions than did the young group (solid line with dots). However, the administration of the physiological stress (pyrogen) lowered the filtration fraction so that at the end of the experiment all three age groups had practically the same filtration fraction. I am intrigued by this finding since our general philosophy has been that the application of a physiological stress would increase age differences. Here is an example where the stress obliterated age differences. Apparently we cannot generalize

From our observational data we have computed renal vascular resistances using the formulae of Lamport (23) Although these formulae involve many assumptions, and we accept them with reservation (25), they are as good as any we have available and lead to some interesting deductions Figure 3 shows the results of these calculations for afferent and efferent resistances The curves show, first of all, a significant drop in both afferent and efferent resistances in the old subjects (dotted line with open circles) following pyrogen administration There was a small but significant change in vascular resistance in the young subjects (solid line with dots) It also shows that there was a *greater fall in afferent than in efferent resistance* Of even greater interest is the increased resistance observed in the old subjects under resting conditions That is, the old subjects show a greater renal vasoconstriction than do the young, even though the old have fewer vascular circuits left in the kidney through which blood can pass This increased vasoconstriction in the old can, however, be lowered by pyrogen From these experiments we have inferred that the renal arterioles in the aged kidney are quite capable of dilating It is concluded that the reduced renal blood flow observed in the aged is at least in part reversible and therefore not the result of structural changes in the renal blood vessels alone

I wonder whether a similar approach could not be used in the study of changes in blood flow induced by vasodilators in other organs I am very much interested in Dr Steele's observations that blood flow

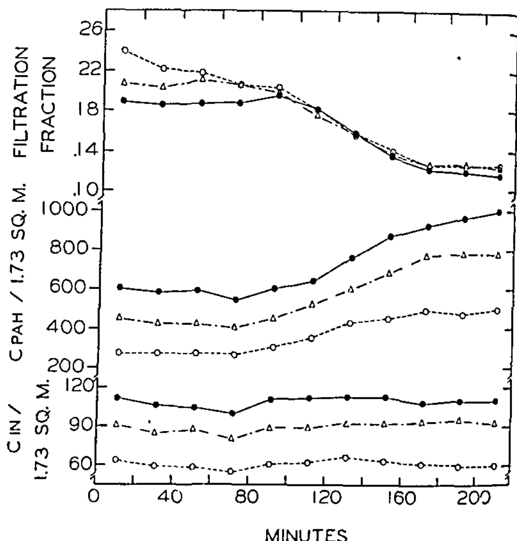


FIGURE 2. Changes in C_{IN}, C_{PAH} and FF during pyrogen reaction.

Fifty million killed typhoid organisms were injected intravenously at O time
o- - - - -o Mean value for 14 subjects in O group (70-85 yrs)
△— —△ Mean value for 20 subjects in M group (50-69 yrs)
●———● Mean value for 20 subjects in Y group (20-49 yrs)

slowly throughout the 220 minutes of observation. It should be pointed out that a rise in body temperature was prevented by administering aminopyrene to all subjects prior to the test.

Figure 2 shows the change in glomerular filtration (inulin clearance), effective renal plasma flow (PAH clearance) and filtration fraction (Cl_I/Cl_{PAH}) following the administration of pyrogen. Although there was no change in glomerular filtration rate, all subjects

showed a significant increase in effective renal plasma flow. The absolute increase was not as great in the old subjects as in the young, but on a percentage basis the increments were of the same order of magnitude (Y = 71%, M = 86%, O = 91%).

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I wonder whether a similar approach could not be used in the study of changes in blood flow induced by vasodilators in other organs. I am very much interested in Dr Steele's observations that blood flow through the skin can be increased as much in the old as in the young.

Steele: In certain ways, arteriosclerotic individuals are more reactive, and seem to me, again, to be comparable to your studies on kidneys.

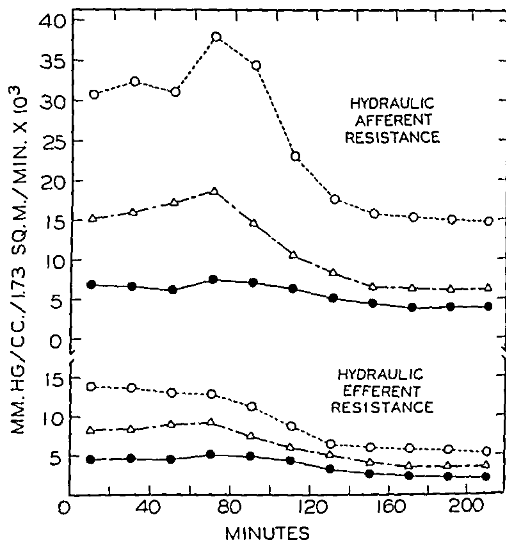


FIGURE 3 Changes in afferent and efferent renal resistances during the pyrogen reaction

Fifty million killed typhoid organisms were injected intravenously at O time

o - - - - - o Mean value for 14 subjects in O group (70-81 yrs)
Δ— —Δ Mean value for 20 subjects in M group (10-69 yrs)
•———• Mean value for 20 subjects in Y group (20-49 yrs)

At rest, they appear to have somewhat increased resistance, but the minute you put them under stress—the dilatation seems not to be less and so the actual rise in blood flow *is greater in* old persons than in the younger ones under standard conditions of *humidity and temperature*. I think that these observations mean just what we said before; arteriosclerosis does not affect those vessels that have to do with the regulation of the *circulation* at its peripheral end, i e., the flow to organs that regulate *metabolism, temperature*, and nutrition

Engle: I was thinking of that, Dr. Steele, in relation to these eleven patients that you have been following for so long, and thinking of it a little bit in reference to what Dr. Shorr and his co-workers have been doing (33). Do the VDM and VEM factors that regulate the number of capillaries that are operating at any time, of the volume of the capillaries—have any relation to your eleven men as regards arterial change and the relation of the gross calcification, the atherosclеromatous condition you get in the big arteries, as related to the metarterioles and the capillary bed?

Steele: Well, of course, Dr. Redisch has for years been following the capillaries under the microscope in arteriosclerotic people, and there is nothing to be seen Their capillaries and precapillaries in the main seem to be normal The usual number of them are open. The others can open up capillaries if you give them the Landis test. Landis was the first that I recollect to use heat as a quantitative test of the vasodilator mechanism (24). They can open up just as many capillaries as a young person and the capillaries do not look very different. They may have a little more tortuous course, but the surprising thing is the lack of difference in the young and old in the tiny terminal arterioles

Of course, those vessels to and from the glomeruli in the kidney are really of a larger caliber than the ones I am speaking of, and they have highly specialized functions Specialization is one of the troubles. There is a highly specialized group of vessels in the fingertips that appear to have more to do with heat regulation than they do with any metabolic function

Shock: What puzzles me is why it is that in a kidney that has lost up to 60 per cent of its available nephrons (25) should superimpose a vasoconstriction to restrict further the amount of blood going through that organ Does that mean that the blood is needed somewhere else or is that too teleological?

Steele. I can't answer that now, but I hope that we will someday have answers to it because we are now set up in a very well-built constant-temperature room and will be able to measure the changes in the kidney, in skin, and perhaps in the muscle, by differential plethysmography We hope to measure changes in behavior to various vasodilators to see what the partition of blood is to the various areas, in the light of whatever arterial pressure changes occur with these dilators It is going to be a hard job to carry out all those measurements at once

One of the things that has come out of our study is how long it takes people to come into equilibrium with the temperature of their

environment. We were in the habit of thinking that you could carry out tests after a half-hour or an hour. Under what are termed "conditions of comfort," equilibrium with the environment rarely occurs before three hours. Only then can you have reasonable assurance that the trend of the blood flow in the extremities will continue to be constant unless it is interrupted by injection of a drug or some other procedure.

Skin temperatures in old people level off at standard conditions —just below those of comfort, roughly, 20° C. with 60 per cent humidity, at a somewhat lower temperature than do the young ones. Put them under stress, however, and their blood flow will rise to exactly the same degree as the younger persons. I think their leveling off at a lower temperature is just what the kidneys did in your group until you stimulated them with pyrogen.

Stern: How far are all these physiological changes actually correlated with morphological changes?

Steele: I think that the lack of correlation is what I was trying to emphasize. Certainly, when you try to relate failure of blood flow to the gross changes in the arterial system, there appears to be no relation Calcified arteries behave as well as regards the amount of blood they can adduce for an organ, or for an extremity, as vessels that have no gross evidence of calcification.

I suspect that the best way to go about studying arteriosclerosis, even though it is the one that is most active at the moment, is from a metabolic point of view. There are many ways of approaching it. There are many things in recent work that give real food for thought

I would like to say just a word about the recent advances. All of us know Gofman's work, of course, with the macromolecular lipoproteins (13, 14). In the first place, from Dr Cohn's work in the separation of the lipoprotein fractions, it looks as though all of the lipid material in blood is associated with protein; that practically none of it is free. Gofman has shown by ultracentrifugation, that if the serum is given the proper specific gravity, lipoproteins float instead of sink. He can on this basis separate them in an analytical ultracentrifuge These normal lipoprotein molecules move at a rate designated in terms of a Svedberg constant as a flotation rate, of 7 to 10 units. In rabbits that have been fed cholesterol and developed atherosclerosis, he has demonstrated the appearance of another group of somewhat more rapidly moving molecules, which have a higher proportion of lipid material than the normal ones.

There seems to be a whole family of larger and larger molecules that move with increasingly greater speed, but he has studied those

of the S_f 10 to 20 group. These he has correlated with the development of atherosclerosis in rabbits and with human groups that may be expected to have arteriosclerosis. One of the groups had coronary thrombosis, and he could show that there was an extraordinarily good correlation with the presence of this condition.

In his general expression of opinion these macromolecules have to do with the cause of arteriosclerosis It seems to me that the appearance of these larger molecules could just as well be interpreted as an effort on the part of the body to retain lipids in stable solution in the blood and prevent their deposition in the artery wall. It might be a defensive instead of a causative mechanism However, the whole field is a remarkable one, and I believe Dr. Simms is collaborating with Dr Gofman to study some of the properties of the substances he has been studying in tissue culture (34).

Simms We have a three-cornered project laid out and all ready to start on, in which Dr Barr and Dr. Gofman and I will make tests on the same samples of serum Dr. Gofman will study the S_f 5-10, 10-20, and 20-40 fractions Dr. Barr will study the lipoproteins and cholesterol precipitated as different serum protein fractions, and at the same time I shall observe the lipfanogens (free, bound and total) and the antilipfanogen (free, bound and total) We hope as a result of this study to find out which of the agents being worked on in each laboratory correspond with agents being observed in the other two laboratories. What the result will be, I can't say yet However, it might be pointed out, that Dr Gofman's S_f 10-20 fraction increases under conditions in which arteriosclerosis is prevalent, while in studies we are making, the agents that increase are the free lipfanogens, the precursors, and the theoretical complex that may exist between the serum lipoids and the antilipfanogen Unfortunately we have no test for this theoretical complex

I might make that a little clearer to those who are not familiar with the work The lipfanogens are substances in blood plasma that cause fat deposition when in a free state (34, 35, 36, 37, 38) Blood plasma also contains an antilipfanogen which prevents fat deposition by combining with the lipfanogens to form an inactive complex ... evidence that ...

lipids, or

tie it up ... activate the lipfanogens.

Steele: Of course, the whole thing fascinates me ...

view ...

least ...

similar

environment. We were in the habit of thinking that you could carry out tests after a half-hour or an hour. Under what are termed "conditions of comfort," equilibrium with the environment rarely occurs before three hours. Only then can you have reasonable assurance that the trend of the blood flow in the extremities will continue to be constant unless it is interrupted by injection of a drug or some other procedure.

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years in Shanghai, told me that the incidence of arteriosclerosis at autopsy is very low among the Chinese.

Steele: I think it is probably a fact, but the point is, even with the knowledge that there is a great difference in the incidence of arteriosclerosis, it still seems necessary to try to document clearly the evidence as to whether it is a racial characteristic or whether it is due to the way you live

Fremont-Smith: Certainly, a comparative study, for instance, of the different racial groups in this city, of which there are a great many, would be much more worth while if you had good statistics and specified statistics as to the same racial groups in their normal habitat. There *is a very real problem here,* because I think you can add up a very large number of diseases which are practically unknown in China, according to what has been said For instance, schizophrenia is unknown, pernicious anemia is unknown, and now arteriosclerosis is unknown, and coronary disease is unknown

Engle Dr Fremont-Smith, is it unknown or undiagnosed?

Fremont-Smith: That is the point I am trying to make.

Engle: Were these data from Oriental medicine, I would be very skeptical about them. I had to go all through that a good number of years ago in relation to prostatic hypertrophy It was alleged that the Orientals had a very low incidence of prostatic hypertrophy. When you get down to the data, there is no such thing. It is a question of reporting

Stern There is a report by Schaltenbran, some years ago, on his visit to mental hospitals in China He saw the same number and proportion and incidence of schizophrenia as in the West, and so did Kraepelin on his original journey to the Orient.

Fremont-Smith: Well, maybe if a comparable study could be made it would isolate one or two points on which there is a real differentiation, which would be very important to know.

Stern But in the question of cardiovascular disease, isn't the cultural pattern, the philosophy of life and so on, the speed of work, actually very important? —so that if you compare Chinese in New York and Chinese in the interior of China, they are entirely different people.

Fremont-Smith: That *is* just the point.

Steele· I think that can be determined. If you really picked your groups well enough, you might come a long way toward knowing whether the development of arteriosclerosis was a racial characteristic or whether it was the result of the way you lived I was impressed with the 13 Chinese in Goldwater Hospital who had marked arteriosclerosis, and I think further study would be *helpful* I am interested in this

plex association of proteins and lipids in the brain are called "proteolipids" instead of lipoproteins, because it is thought that the proteins are on the inside of the molecule. This view seems to make good sense to the electrophysiologists because if the nerve sheath is plated with a polar and nonpolar molecule right together, it accounts for some of the characteristics of the electrical transmission along the nerve.

If you don't mind, I would like to return to what you think about the possibility of studying the occurrence of arteriosclerosis in different racial groups. Even here in the city of New York, I think it would be helpful to study groups that apparently have differences in the amount and degree of arteriosclerosis and to learn enough about their habits and customs, chiefly, diet, to decide whether racial characteristics or way of life was more important.

Engle: Before you go into that, I have a note here to ask you to go a little further and discuss the statistical problem as you see it for the Orient. I was impressed with it a couple of years ago when I was on duty at Hiroshima and watching the diet of the poor Japanese. I thought that was a perfectly wonderful place to go and see whether low animal protein and low animal fat in the diet had anything to do with general cardiovascular-renal hypertensive disease. What do you think about the statistics from those countries? Is there less disease than in a general population like ours?

Steele: Well, I don't know. I suspect there is, but that is just the point I should like to make; I think studies in the various countries must be made, if not by the same people, certainly by a central control agency so that the ages, the circumstances, and the techniques for making the observations are identical.

Now, everybody who comes back from China writes in an offhand fashion that they saw only one coronary thrombosis the whole time they were there. They do not bring back figures for the age groups seen. Therefore, it seems to me that until studies are carried out by identical techniques we will get nowhere. The welter of reports that are brought back piecemeal do not help much

Shock: Does anybody have data on autopsy reports on Chinese comparable to the kind of thing you get in the German literature? I am thinking about the reported difference in the incidence of sclerotic changes between the North Germans and the Viennese. I think there was at one time a belief that those who had a lot of cheese and milk products in their diet showed a higher incidence Am I correct in that, Dr. Goldzieher?

Goldzieher: Well, so far as the Orientals are concerned I have only hearsay knowledge. My friend, Dr. Frederik Reiss, who spent fifteen

interested in this area, but I don't think that we should dismiss too readily the idea that they could be interested. As a matter of fact, it has been pointed out by Dr Chisholm that the World Health Organization, as in the case of the other specialized agencies of the United Nations, is very responsive to recommendations coming to them from international voluntary organizations in their particular field or area, and that they can be stimulated to take action in an area for which they have responsibility if an international voluntary organization says, "Look here, you should pay more attention to . . ." Therefore, I bring this up at this point, Dr. Cowdry, because of the forthcoming International Congress on Gerontology, which might very well make a recommendation to WHO, that here is an area in their general purview which would be important to know more about.

Cowdry: I agree with you that they should and that it is a healthy thing for [illegible]
mindful [illegible]
the geogr[illegible]

Fremont-Smith: Did that come to them from an international organization?

Cowdry: Yes, to some extent

Fremont-Smith: I mean, a specific recommendation? If you wanted to get them to take new action, it was necessary for them to have the recommendation come from an international organization. I have a very vivid example When the International Congress on Mental Health and the forthcoming World Federation for Mental Health made the specific recommendation that WHO should set up a section on mental health, it responded and stated that it had done so as a result of this recommendation Therefore, I think it would be worth while to consider making a specific recommendation to them. Perhaps this group itself might informally suggest that the International Gerontological Congress take this up and see whether the WHO is interested Although we are not an action group, it could come out of these discussions in an informal way, or be brought to your attention, sir, as President of the Congress Certainly no harm would be done by a strong recommendation to WHO

Cowdry I am all for giving them an opportunity to do something worth while, and I think we ought to phrase this request very carefully in terms of a concise statement as to what we want them to do.

Fremont-Smith: I think perhaps it could be proposed to the forthcoming Gerontological Congress Perhaps we can get the thing started informally because we tried very hard not to have any of the Foundation's conference groups act as action groups in any sense

subject and have been talking it over with the New York Heart Association. We were wondering whether some studies of this nature on the various racial groups in New York City would be worthwhile.

Goldzieher: I don't think we can dismiss these differences by assuming that they were not properly diagnosed. Leading German pathologists who have travelled abroad were so impressed with the geographical variations in the frequency and character of certain diseases that just prior to the onset of World War I they were about to found a Society for Geographical Pathology. That there are such differences I can testify myself for I had the opportunity to compare the pathological material studied in Central Europe with the autopsy material I have seen here in the City of New York and I must state that certain conditions which are extremely rare in Central Europe are rather common here, whereas others which we did see over there quite frequently are rare here. I may mention as an example Gaucher's disease which, looking back at my old material of about 20,000 autopsies, I have come across only once with two surgical specimens added. That was three cases (including the surgical material) in a population of 20,000. In New York, on the other hand, in about 1,500 autopsies I saw 7 or 8 cases of Gaucher's disease As this could not be a question of diagnosis (the observer being the same), it must be due to the greater frequency of the conditions in this geographical area. Several other pathological conditions could be mentioned, the occurrence of which is similarly variable.

Cowdry: Is there any organization that would be interested in such a study as there is an organization which is interested in the geographic distribution of cancer?

Frank. The World Health Organization.

Fremont-Smith: There used to be a National Geographical Diseases organization, I believe, or an International Congress of Geography of Disease

Cowdry: That is the one that McKinley was interested in, but the movement has not progressed very far

Fremont-Smith: No, I think Mr. Frank is right; that the World Health Organization should be very definitely interested in this area.

Cowdry: I don't think the World Health Organization would be, because this is not a problem where you can, by use of some drug such as quinine, or by some means of diagnosis, influence large numbers of the population for the better. This is a problem to discover the frequency of something that probably may be very difficult indeed to treat.

Fremont-Smith: I believe that they have shown evidence that they are interested in basic frequency problems. I am not sure they are

also make note to what extent the data coming from any country fulfill the criteria? I think you are right, Dr. Steele, that it is going to be a long-time affair before we will have comparable data, just the bare, raw data, and it will be a much longer time before we can get comparable data under comparable cultural and dietary conditions. That is another issue, and a very difficult one. But just raw data on age groups, of autopsy material, in themselves would be a starting point, because it would immediately call attention to gross discrepancies which could then be explored as to their meaning

At the present time, it seems to me, we don't have raw data for many of the countries for which we need them, and that is the kind of thing, for example, that WHO can call to the attention of the countries involved, and that is one of the means by which they gradually build up the capacity and criteria within those countries for the collection of their own data. Then WHO constantly sends teams around from time to time, and in that way they also raise standards But it is probably true that you couldn't get a large enough team together to go in and spend a number of years in a country doing the autopsies themselves. It would mean a gradual raising of the educational standards, the competence, and the criteria of the people doing the autopsies

Oliver There must be records at Peking or Hsiang Ya Medical School, Chang Sha, where for a great many years, there has been careful supervision This autopsy material would give the incidence of arteriosclerosis

Shock· What about Hawaii as a source of reliable autopsy data? There are groups of Chinese and Japanese there, I understand, with medical records Would the records of that population offer any useful evidence?

Steele: W [illegible]
on studies [illegible]
here in the [illegible] its extraordinarily diverse population.

Simms You would be more apt to get uniform diet in New York, although even here, they eat different foods.

Engle· The nisei and issei eat different foods from the first generation that arrived here

Shock· I think that was shown very strikingly in the growth studies that were done some years ago by Preston (31) on the growth patterns for Chinese and Japanese children living in San Francisco. You recall that the Chinese children in San Francisco were about the same height and weight as Chinese children from the area in China from which their parents had come, whereas the Japanese children were a couple

Cowdry: If we really want to get action by WHO, the Gerontological Society, the International Gerontological Congress, this Conference group, the Society for the Study of Arteriosclerosis and all the other interested groups should likewise propose it to the Congress in order to get the weight of scientific opinion behind it. This would strengthen the recommendation of the Congress.

Fremont-Smith: What we are really after here is to get an expression of opinion that a study of the geographic distribution of disease, and particularly of arteriosclerosis, is something which we need for the furthering of our research. That is all we need to state, just the way you stated it, Dr. Cowdry, and if there is general agreement to that as a proposition, that can be picked up. I think we would not address it to anyone.

Engle: I think the major difficulty there is, perhaps, just in the technical competence of the people in these countries who are to do the work. The rarity of autopsy material in the Japanese hospitals is something that completely astonished me; that is, since the war and under American occupation. I have not worked in China but, certainly, outside of the larger centers, I would suspect the same thing to be true. It would take a complete team, set up to do the job by our own standards and methods, if comparable data are to be accumulated

Steele: I think that is right I don't want to belabor this, and I appreciate, Dr. Goldzieher, that there are certainly geographical differences, but to follow them up with proper data so you can sort out the environmental and hereditary influences is necessary The data have to be exact and comparable Two years ago when I spent three or four months in the Philippines, I was told there was almost no rheumatic fever there and very little arteriosclerosis and hypertension Well, I don't know about arteriosclerosis because I didn't have very good means of documenting it But, certainly, the amount of hypertension was incredible Dean Sison of the University of the Philippines has been assembling data on rheumatic heart disease for the last three years, and it is clear that they do not seem to have attacks of acute rheumatic fever and they almost never see a rheumatic nodule But the amount of mitral disease is quite comparable to that of the Middle Atlantic states, according to the material in the Hospital of the University of the Philippines. That is the sort of thing which makes one doubt how much one can rely on the information obtained unless one does just what Dr. Engle said—assemble a team that is going to get comparable and intelligible data.

Fremont-Smith: Isn't the World Health Organization, better than any other, the one that can set up criteria for such a study and then

to collect simply what is known before setting up an elaborate scheme. It is not known how much information is in the records at Peking. I doubt whether you can get at that information now.

May I turn back to something for a moment? I was very interested in Dr. Stern's remark about the lack of correlation between cerebral arteriosclerosis, if I understood him correctly—

Stern: That's right.

Steele: —and senile psychosis. I haven't had much experience with it, or probably not as much as he has, but I have been impressed by the fact that whether there was marked cerebral arteriosclerosis or none, you could still have behavior patterns that are diagnosed as senility

Stern: Well, of course, in our experience, absolutely no conclusive, systematic work has been done as yet but there are two different entities; that is, senile dementia and arteriosclerotic psychosis. The main point, clinically, is that in senile dementia you have the so-called organic syndrome. By that, we mean impairment of memory, disorientation, deterioration, and emotional fluctuations The whole clinical course shows a general down-hill course with minor fluctuations [illegible]

For instance, you may have sudden changes in behavior I remember one patient who was sitting in the admitting office of a mental hospital. He looked completely well He was rational, he was well oriented, and he was neatly dressed The commitment papers seemed to belie his state They spoke of a man who undressed in public, who was confused and noisy, and so on We almost thought there was some foul play in the admitting papers, but the same night, this man relapsed into that psychotic state You would not find that sort of thing in a so-called senile psychosis

From the pathological point of view, in senility of the brain, there are two different types or, actually, two different trends, one might say, in the morphology First of all, in the senile brain, you have changes which are characteristic of any senile organ; that is to say, increase of lipochromes, the so-called brown pigment, and then, a diminution of parenchyma and a relative or absolute increase of interstitial tissue. In addition to that, you have something in man which, very strangely, is typical of the human brain and apparently does not occur in any other species in old age, and not only that but it is characteristic of phylogenetically recent areas of the human brain, and those are changes of

of inches taller. When they examined the data, they found that the Chinese in San Francisco live in pretty much the same cultural pattern and eat the same foods as they did in China, whereas the Japanese who had moved in had shifted over entirely to American diets. They were eating Wheaties for breakfast and following the American dietary pattern, and it showed up very, very markedly in the growth rates of the two groups.

Hamilton: This may not be appropriate to the World Health Organization, but with reference to racial differences in some diseases, we have a bit of data, and since maleness phenomena are, I suppose, of some interest in early coronary thrombosis and such, it might be well to bring them up now.

We have data for three things: baldness, acne, and color blindness. Our data are from New York Chinese and some Pawnee Indians who are supposedly as full-blooded as any Indians in this country now are. The incidence of both baldness and acne is considerably lower. The occurrence of color blindness is also considerably less frequent.

Steele: In the Chinese?

Hamilton: Yes, in the Chinese. It is lower than for the American population; so there are racial differences, to be sure, in those three things in people in this country.

Fremont-Smith: So far as I know, and I may not know but I believe, the Public Health Service is not restricted from getting information in other countries, provided the information is needed for research being carried on in this country.

Cowdry: The Public Health Service makes grants directly to other countries.

Fremont-Smith: I know it does, but it does so under the restriction that the grant must be for the purpose of research, which will be of some direct help to research carried on in this country. Therefore, it seems to be entirely possible that this suggestion could be brought informally before the Heart Institute and, if it is of sufficient significance, a grant might be made to assist some of the work. For instance, there might be an exchange program, like that you were speaking of, Dr. Engle, to bring back from other countries the data which would be helpful to our own members working on Public Health Service grants.

Cowdry: The cancer group has done just that. It held a meeting in Oxford last summer and recommended the establishment of a unit which is now centered in the National Institutes of Health just outside Washington. Active studies on the geographic distribution of cancer are going forward. I wonder whether we couldn't follow suit.

Steele: Dr. Cowdry, your suggestion is a wonderful idea, starting out

... the minor microscopic changes have the same degree of arterio-
The only thing
the relationship
between the parent artery and the artery which penetrates into the brain; that is to say, in the arteries which supply the hypothalamic area and the quadrigeminal above it, you have many extracerebral anastomoses and you have a gradual diminution of the size of the artery from the large artery down into the area of the brain, whereas in the vessel supplying the internal capsule you have the middle cerebral artery and immediately, without any transition, one, long, penetrating artery Now, whether that is really the explanation, I don't know, and I would like to hear comments on this problem.

Engle: Dr. Stern, you have introduced much new material with your remarks I am delighted with it, and I am only sorry that we don't have a specialist in neuropathology present, but we do have present a man who has been persistently silent all day, a very well-known pathologist Dr Oliver, would you pick up this problem?

Oliver: The reason why I am often silent is that although I do have certain impressions that might be offered in answer, since I have not specifically examined the problem it is a very general impression that I have to draw on

Now, certainly, as Dr. Stern said, this lenticulostriate artery—I think the anatomists have even given it a name, haven't they?—

Stern Yes

Oliver. Probably because it is so frequently involved as to deserve special designation

There is no question of the fact The usual explanation is the one Dr Stern has suggested. that the artery comes off from its parent vessel at a right angle and as there are no other branches to take the strain of the blood flow, *it suffers* All this seems quite logical. But if that explanation takes care of one of the mysteries, there are similar ones in other places For instance, why do plaques form around the orifices of the intercostals? The old idea was that the blood flow was turning a sharp corner at this point, and so was impinging on the wall and that, therefore, it was again a matter of mechanical strain. Dr Wilens (41) has introduced a much more subtle explanation and perhaps a better one; namely, that at the exit of the intercostal artery the wall of the aorta is tied down by connective tissue but is more or less loose elsewhere When pulsations ...
of the intercostal is therefo
fore anything that is being ... such as fat,
will simply shift down to the nodal point of rest and remain there It

the neurofibrils inside the nerve cells which form these beautiful, regular networks normally, but become very coarse and come together and, in the severe cases, become curled up in an irregular form. These changes and these peculiar plaques of argentophile material, the origin of which we don't know, occur only in the cortex, and particularly in phylogenetic areas of the cortex. They look like artefacts, if you are not used to them. These two features are apparently specifically human and do *not* occur in other animal species. In animals who become old in wild life, they are also not found. Now, these changes, plus the ordinary senile changes of the brain, are completely independent of the state of the blood vessels. On the contrary, there seems to be almost an inverse relationship, so in most cases where you see very marked changes in the neurofibrils, the vessels look like vessels of young people.

In arteriosclerotic brains, you do not find these changes, as a rule Clinically, too, there is a difference, as I said. If we had an effective therapy of the sclerotic brain, I think the differential diagnosis would have been cut finer than we can do it so far

There is one point I would like to bring up for discussion and ask the pathologists about it; that is, in arteriosclerosis of the brain, there is something which we call local vulnerability The tendency to succumb to ischemic or hemorrhage necrosis or massive apoplectic hemorrhage is not evenly distributed in the central nervous system. If arteriosclerosis is ubiquitous or if the pressure within the arterioles were the same all over the brain, you would expect hemorrhages to occur with the same frequency, distributed according to chance But, as everybody knows, the hemorrhages, not only the massive apoplectic hemorrhages but also what one usually calls thrombosis—that is to say, the ischemic and hemorrhagic necroses are more frequent in certain areas than in others. For example, there are arteries which ascend from the middle cerebral into the internal capsule, where hemorrhages occur with increased frequency, and there are other areas which are extremely resistant to hemorrhage For example, even if you cut serial sections, you will hardly ever find ischemic areas in certain parts of the hypothalamus or in the quadrigeminal plate I recall only one case I have ever seen where there was an ischemic area in the quadrigeminal plate, and there I am not even sure whether it had anything to do with arteriosclerosis.

We did a study many years ago, in which we compared the degree of arteriosclerosis in these susceptible and resistant areas The peculiar thing was that there was no difference in the degree of arteriosclerosis between the two areas. In other words, the arteries which supply the quadrigeminal body, which hardly ever show any apoplectic changes,

pressure is low, there is quite a tendency for hemorrhage, and in shock there is an excess of heparin in the blood. I think, perhaps, that in blood vessels you have something like a self-sealing inner tube of an automobile tire The living cell is that way. It seals itself when it is broken, and this sealing mechanism is related to blood clotting. With an excess of heparin, there may be a tendency for blood to escape when, ordinarily, at the beginning of such an escape there would be a clot that would seal it in One can think of it that way or one can think of some substance in the blood vessel wall as being hyaluronic acid, and it may be that some of the heparin-like substances that really are present in the blood in shock tend to open up the blood vessel so it is more sieve-like. It may be a naive idea, but it has been appealing to me

Horvath: However, you can keep an animal completely heparinized for many days without seeing loss of blood or fluid through the vessel walls Even in those animals where you raise the blood pressure also, there is no break in the vessel wall or escape of material. Of course, loss of fluid will occur if anoxia is present in the small vessels. Anoxia might be a better explanation.

Heilbrunn: No, let me explain Heparin is not a single substance. There is a large class of heparin substances. We have been working with them, and some of them act very strongly on the blood and not so strongly on cells We have been interested in the effect of heparin and of [illegible] has an ef[illegible] poly-sa[illegible] This bacterial polysaccharide doesn't work strongly on blood clotting but it works strongly on protoplasmic clotting It also causes quite a lot of bleeding as well. As a matter of fact, heparin itself sometimes does cause hemorrhage when used clinically

Stern May I pursue the discussion of cerebral arteriosclerosis a little further?

Engle. Please do

Stern: A great source of confusion in most discussions in the literature on the pathogenesis of these apoplectic lesions in the brain is due to the fact that some authors seem to confuse the hemorrhage infarct, which is actually only a modification of a necrosis, with apoplectic hemorrhage

There are two types of brain hemorrhages in arteriosclerosis. one in which you have an ischemic necrosis and you may or may not have hemorrhages into the ischemic area, which often are confluent, so that they give the impression of a massive hemorrhage. However they have a tendency to be confined to the gray matter and very often imitate gray matter and will stop short, for example, in the caudate nucleus

might be that some such mechanism is operating in the cerebral artery.

I have had Wilens' theory amusingly demonstrated in my house. There is in it an attic with a plastered ceiling which is supported by stringers that come down in pairs from the roof. On going up there one day, I saw that a deposit of dust had produced two rows of spots along the length of the ceiling with relatively little elsewhere. I was completely mystified as to why that dust hadn't spread all over the ceiling until I remembered the pattern of interiosclerotic plaques in the aorta and realized that the points of dust deposit were where the stringers came down and fixed the ceiling so that it could not vibrate. In other words, if one made a picture of the vibration of the ceiling, the nodes would be at the point where it was fixed and therefore the dust would naturally stay where it wasn't disturbed. Perhaps some such mechanism as this operates in the pulsating artery; where it is fixed, fat deposits and the fatty spot is the place that usually breaks.

Stern: There is another possibility. This artery is also one of the predilective arteries for embolism, and the explanation is that the embolus, on its way from the heart, takes a pathway which has the least angles Some people say, therefore, that perhaps in apoplectic hemorrhages, there is something like a wave going along the arterial tree which also will have its greatest impact in that particular area. We see, for instance, that those areas which are highly resistant to arteriosclerotic changes, like the spinal cord and the quadrigeminal body, are also extremely rarely affected by embolism.

Horvath: Isn't this situation somewhat analogous to what happens in coarctation of the aorta, where you have a very nice calcified plaque at the point of coarctation, yet a hypertension above, which is almost sure to be a mechanistic type of hypertension, and when you remove the coarctation, the hypertension in the upper extremity disappears and yet there isn't a fixed point there? That is, it isn't fixed in the sense of the same thing that we see in the intercostals

Oliver. The coarctation might be fixed by fibrous adhesions.

Horvath· It is relatively fixed, yes, but it is a point at which you have a high level of pressure, with practically no outflow from the end of it, in other words, it is the same thing as where you have a right angle—well, not a right angle, but it is essentially a right angle so far as this is concerned.

Heilbrunn: May I say a word out of turn? I am not a pathologist and I know nothing about pathology I have come to think that hemorrhages are sometimes caused not by excess pressure but by a dissolving out of something in the walls of the blood vessels I have come to think in this way because in shock in which, as everybody knows, the

pressure is low, there is quite a tendency for hemorrhage, and in shock there is an excess of heparin in the blood. I think, perhaps, that in

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and again start in the lenticular nucleus, whereas the internal capsule may be comparatively free.

The other type of brain hemorrhage in arteriosclerosis is entirely different from the standpoint of pathogenesis. That is, the so-called massive apoplectic hemorrhage does not follow any distribution at all in the actual structure of the nervous substance, but it is usually egg-shaped and looks like a violent eruption from the artery into the tissue. I think that the pathogenesis of these two things is entirely different.

There were two Germans about fifteen or twenty years ago who did a very peculiar study in which they injected material into corpses with arteriosclerosis, under high pressure, at one and a half atmospheres, and they were not able, with this high pressure, to have actual leakage from arteries into the brain. In other words, the textbook tradition that these arteriosclerotic hemorrhages, these massive apoplectic hemorrhages, are due to a sudden increase of intra-arterial pressure may not even be true. Your explanation of the role of heparin and so on may perhaps be correct in these so-called hemorrhagic infarcts. But these massive apoplectic hemorrhages which are so explosive, so to speak, are an entirely different type of bleeding. I wonder how you feel about the pathogenesis of that, Dr. Oliver.

Oliver: I have always thought that there might be two sorts of intracerebral hemorrhage; one as you described it, beginning as an infarct or a softening of brain substance into which bleeding occurs, and the other a simple blowing out of an arteriosclerotic spot in a vessel, so that the blood escaping under high pressure causes destruction of nerve tissue There have been those who have claimed that all apoplectic hemorrhages are parenchymatous; that is, that there is tissue damage, a softening perhaps, first and that then hemorrhage occurs secondarily into the softened areas. This never struck me as very plausible. But here, again, I haven't given these matters anything more than the usual attention that the general pathologist gives them, and so my impression might not stand up to critical examination.

Shock: Have any observations been made on cerebral blood flow in patients with arteriosclerosis?

Horvath: We did a few at Pennsylvania but not enough to make any real analysis. It doesn't appear from the few that we have done that there is a great deal of difference in cerebral blood flow. The difference is primarily in the cerebral vascular resistance, which, of course, goes very high (18). But as far as the actual amount of blood going into that area is concerned, it is still practically the same; it is still in the neighborhood of 50 to 60 cc. per unit of mass, per unit of time. The other thing we found was the decreased utilization of oxygen by

the brain That, I think, is probably a more important finding than is the fact that the blood flow is not altered. In other words, as the blood goes through the area, there is less oxygen taken out than is usually the case. We have not studied enough cases yet to draw definite conclusions, but it appears that there is some evidence of ineffective utilization of oxygen by the brain in arteriosclerotics

Shock: But there was not a very large range in the change in blood flow with changes in the state of consciousness, either, was there?

Horvath. No, absolutely not (19) The only conditions in which we found marked changes in blood flow were very deep depressions with anesthesia (21). But we studied quite a number of cases of schizophrenia, and there is no apparent difference in those individuals compared to normals (17, 21), so that it is a little difficult to say that in this whole mass the blood flow is altered, but simply there may be some alteration in the utilization of oxygen, and that is the problem we have been working on

Heilbrunn: Do you think that is cellular?

Horvath: I have no idea. It probably has to be, basically.

Steele: In the first place, how does cerebral blood flow *remain* unchanged and yet the resistance go up without a comparable change *in* perfusion pressure? You have to have tremendously high blood pressure, haven't you?

Horvath: Well, you do and, relatively speaking, the blood pressure level is up, isn't it?

Steele: In all of these persons [illegible]

Horvath: Well, in the patients t[illegible] pressures have been *in* the neigh[illegible] mercury, which is certainly much higher than the 85 mm. Hg that we have found in the normal young adults that we have studied. These pressures may *not* be as high as those observed in certain cases of hypertension, but they still are high That has to follow, you see.

Steele: Yes, it does, but so many of these arteriosclerotics that I see have perfectly normal pressures It must be a specialized group you have seen with high pressures

Horvath Well, of course, those are the ones we get to study first, as you well realize It isn't always easy to select your cases.

Steele: Well, another thing is that I have just heard Scott (32) from Blalock's department talk He has done a most remarkable piece of surgical work on coarctation of the aorta in dogs, with a little shunt that he makes out of one of the arteries to the foreleg. Now, when he transplants kidney up above the coarctation, the pressure above the coarctation falls almost more than does the pressure in the legs of those

animals, so I am not a bit convinced that the hypertension of coarctation is a good example of mechanical elevation of pressure.

Horvath: Well, it is one of the possibilities, and certainly—and I haven't seen this particular work of his so I don't really know how to analyze it at the moment, but at least the evidence that is available at the moment seems to indicate that the hypertension in coarctation is closely related to some mechanical factor. There is some work published just recently by Wiggers on the pressure waves in coarctation. It was not a clinical type of coarctation. It was experimental coarctation, which seems to point toward a mechanical type of change. Now, that isn't necessarily so because, after all, the coarctation that we see in man varies extraordinarily from one individual to another.

We saw a patient just a while ago who was 65 and had had a coarctation, noted at age 15 but refused operation Here he was, with no apparent damage despite the fact that his hypertension in his upper extremities had been there for some forty-five or fifty years, whereas there was slight hypertension in the lower extremities and yet there was no apparent damage to that individual in any way. I don't know what we can say about that, except that the data are confusing

Steele: I have been interested in the mechanics of the hypertension in coarctation because it seems to me the best evidence lies on the side that it is renal in origin.

Horvath: I could extend the argument a little bit further As far as we can find out, there is no evidence of renal impairment in these patients.

Steele: That is true You don't have to have diminished renal function to develop hypertension I was interested in the discussion of the localization of arteriosclerosis in the brain because the question is one of general interest Why do the coronary arteries lying on top of the heart become subject to an immense amount of sclerosis, and yet the muscular branches that have comparable pressure by the muscle on the outside, probably with some degree of protection on the exterior surface of their walls, almost never have it?

I come back to what I think is really a beautiful observation by Dr. Dock (6) on the difference between the thickness of the coronary intima, even at birth, in men and women, and the condition that is set up there with loose, thick intima that can be stretched out and then folded back up when the heart contracts Study of factors that go into the localization, I think, is another possible way in which we might learn a good deal more about the actual stress and strain and effect of pressure. I think almost every one of us would agree that elevated pressure is probably not the deciding or primary factor in the produc-

tion of arterial lesions, but it must be a dreadfully important secondary one. You've got to have the high perfusion pressure; maybe also lipids in the proper state or in high enough concentration.

The other experiment that Dr. Wilens did was of considerable interest (42) He maintained rabbits in an unusual position and got fatty plaques deposited in unusual sites. I think the worst lesions moved down toward the lower end of the aorta.

Horvath: Of course, the question is, why do you see more coronary occlusions in the left coronary artery than you do in the right, and yet they are in the same organ and they are under the same surface?

Oliver: Of course, these vessels have a spot of arteriosclerosis on which the clotting occurs, but then the question is simply put back further—Why do these vessels get the arteriosclerotic spot?

Steele: In the primary branches, anyway, Ehrich, de la Chappelle and Cohn (8) showed that in the right and left, and the circumflex in all [illegible] identical. It was [illegible] right [illegible] re in the right ventricle is different from what it is in the [illegible] right and left coronaries originate, obviously, at the same pressure, but beyond the three initial branches differences become apparent.

Engle: Well, let's go back to some of these problems of stresses and strains on this well-known cardiovascular system. Dr. Steele hasn't given us more than a small fraction of his experience and reflections in his presentation on this problem, so, Dr Steele, you pick it up again

Steele: I am about nine-tenths milked dry. I would like, however, to go back to some of the notions expressed about hemorrhage in the brain I think we have, in recent years, too often lost sight of the possibility that cardiac infarction—and it is an important matter in view of the present notions of therapy with the anticoagulants—may in part be due to hemorrhage of the arterial wall The work of Winternitz, and more especially of Horne, whose estimate was that upwards of 60 per cent of cardiac infarcts are at least in part due to some kind of a break in the vessel wall, with hemorrhage, possibly followed by pushing the internal layer of the wall inwards, and final plugging of the vessel with a clot or fibrosis You don't get many surgical biopsies of coronary arteries, I imagine, and at autopsy it is too late to tell what happened

I remember a meeting in Boston where Dr. Herrman L. Blumgart presented the wonderful work on the pig's heart and then showed that humans who have had one occlusion have similar ramifications of the collateral circulation so as to make a complete and final occlusion

animals, so I am not a bit convinced that the hypertension of coarctation is a good example of mechanical elevation of pressure.

Horvath: Well, it is one of the possibilities, and certainly—and I haven't seen this particular work of his so I don't really know how to analyze it at the moment, but at least the evidence that is available at the moment seems to indicate that the hypertension in coarctation is closely related to some mechanical factor. There is some work published just recently by Wiggers on the pressure waves in coarctation It was not a clinical type of coarctation. It was experimental coarctation, which seems to point toward a mechanical type of change. Now, that isn't necessarily so because, after all, the coarctation that we see in man varies extraordinarily from one individual to another.

We saw a patient just a while ago who was 65 and had had a coarctation, noted at age 15 but refused operation. Here he was, with no apparent damage despite the fact that his hypertension in his upper extremities had been there for some forty-five or fifty years, whereas there was slight hypertension in the lower extremities and yet there was no apparent damage to that individual in any way. I don't know what we can say about that, except that the data are confusing.

Steele: I have been interested in the mechanics of the hypertension in coarctation because it seems to me the best evidence lies on the side that it is renal in origin.

Horvath: I could extend the argument a little bit further. As far as we can find out, there is no evidence of renal impairment in these patients

Steele: That is true You don't have to have diminished renal function to develop hypertension I was interested in the discussion of the localization of arteriosclerosis in the brain because the question is one of general interest Why do the coronary arteries lying on top of the heart become subject to an immense amount of sclerosis, and yet the muscular branches that have comparable pressure by the muscle on the outside, probably with some degree of protection on the exterior surface of their walls, almost never have it?

I come back to what I think is really a beautiful observation by Dr Dock (6) on the difference between the thickness of the coronary intima, even at birth, in men and women, and the condition that is set up there with loose, thick intima that can be stretched out and then folded back up when the heart contracts. Study of factors that go into the localization, I think, is another possible way in which we might learn a good deal more about the actual stress and strain and effect of pressure. I think almost every one of us would agree that elevated pressure is probably not the deciding or primary factor in the produc-

know is whether you think there is any relationship between coronary thrombosis and these sludges that Knisely has been studying in the circulation (22)?

Steele: That is a good question I hadn't really thought of it and do not know whether there is any connection between the two.

Cowdry: You can see these sludges, of course, in the retinal blood vessels

Oliver: It would have to be a pretty big sludge to close up a coronary artery.

Cowdry: I want to know about the little sludges that go around, whether they are more frequent in individuals who are likely to get or have gotten coronary thrombosis.

Steele: That would be a good way of studying the subject but I know of no studies so far

Cowdry: I thought the work of Knisely excellent, and it was published at considerable length, you will recall. When you see these masses of red blood cells circulating in small vessels, you can easily imagine that they may have some relationship to occlusion

Heilbrunn: I think it is a clot Knisely, himself, has suggested that sludge might be due to a clotting. I think everyone is pretty well agreed that in the blood there is a constant equilibrium between the clotting factors and the anticlotting factors

Oliver: Is there fibrin in these sludges?

Cowdry That is the point

Oliver: The important question is whether the sludge is an actual clot or just an agglutination of red cells.

Horvath: It is just an agglutination, but there is fibrin in it.

Heilbrunn. Well, Knisely himself thought that the cells might be held together by traces of, say, fibrin.

Fremont-Smith: Isn't there some evidence recently that there are several intermediary stages between fibrinogen and fibrin, or several different molecular species concerned, and that possibly—and this is now my interposition and I don't know that this has been suggested—at the earliest change from fibrinogen towards fibrin, it might be reversible? That would fit in with what you were saying Some recent work came out in our last conference on anticoagulants (10), to the effect that there were several steps in the molecular change from fibrinogen to fibrin

Cowdry. Yes, I think I heard something about that, but I am not sure, either

Heilbrunn: These sludges are inside the vessel, so I don't quite see how it can be proved whether there is fibrin in them or not, unless a

with infarct less probable in the region around it. Fuller Albright asked if he was advocating prophylactic ligation of the coronary arteries in the fifties. Dr. Blumgart did not rise to the amusing side of it.

But it does seem to me that this line of work would be a very profitable one for pathologic study. If it is really true that many closures of vessels begin as hemorrhage, or even if they begin otherwise, hemorrhage becomes a secondary or even a tertiary event, one wonders very seriously about the liberal use of the anticoagulants.

We set out with some such purpose in mind a little while ago, but have not gone very far. A large number of sections of the coronary arteries from about four or five patients were examined In old lesions, however, it is almost impossible to pick out of the pathological picture what occurred first. When one is dealing with relatively recent infarcts and with considerable amounts of blood in the myocardial tissue, one still cannot be certain whether blood got into the area of softening from the ruptured artery or whether it might have leaked through badly damaged capillaries due to anoxia from the sudden plugging of an artery. On the other hand, many of the things you see in fresh coronaries could have resulted from a damaged artery that subsequently was ruptured, particularly from the smaller branches as they begin to dip into the myocardium.

I would like to ask Dr. Oliver what experience he has had with this process In the mad rush to study disease of the arteries metabolically, we may have overlooked some of the prior notions about what might happen to arteries besides lipid deposits. I said before, Dr. Lansing and his group (27) have taken the view, and there is good evidence for it, that chronological changes may prepare the vessel for subsequent injuries.

I don't know whether you have run across the work, but there was an article by Dr. Irving S. Wright, who gathered statistics on the use of dicumarol in coronary thrombosis from many different hospitals and clinics under different regimes, and who tried to collate the data from them (43). Doscher and Poindexter (7) pointed out that age was not well differentiated and that the number with hypertension was not clear, a factor of importance in the likelihood of recrudescence. It was shown that in groups of people with and without hypertension, in males and females and in old and young groups, much greater variation in the rate of coronary thrombosis — much greater intergroup variations — might occur without introducing dicumarol than was shown to be the case in the thousands of individuals that Wright reported.

Cowdry: May I ask you a question at this point? What I want to

and what I always love, are semantic differences "It is not fibrin." "If it is fibrin, it is not real fibrin; at least, it is not the fibrin that I mean." I think we have to say, then, fibrin with respect to what? This is fibrin with respect to irreversibility.

Oliver: Such a meaning has a real, practical importance to the pathologist, at least.

Fremont-Smith: The chemist has a different fibrin which he identifies by its molecular size and shape and weight and other aspects. But on most of these misunderstandings, I imagine if we had the right people here, a terrific row could start up right now "It is fibrin, it is not fibrin" I think that this illustrates the kind of specification needed in scientific discussion I think it is perfectly clear that we are talking about different kinds of fibrin.

Horvath: I have forgotten exactly where I saw this or whom I talked to about it but there [illegible]

[illegible] of the two it is.

Oliver Globulin — if I may add a comment which will clarify at least my concept as to the difference between agglutinated clumps of red cells and clots composed largely of red cells and the relation of these two aggregates to globulin, fibrinogen and fibrin Although these two sorts of aggregates appear remarkably similar to the eye, I *think* there is a very practical and therefore *significant* difference in their make-up

In the agglutinated clumps the red cells are held together by electrostatic forces which in turn are dependent on cell surface layers composed in part of a globulin which may be very similar to fibrinogen. The electrostatic changes on these surfaces vary with the [illegible]

[illegible] clumps may be readily dispersed or again agglutinated. All this has been demonstrated by appropriate physical measurements (29).

In the clots the constituent red cells are bound together mechanically by threads of fibrin. Since the fibrin is an irreversible compound, short of enzyme action, the clot is a relatively permanent aggregate The difference in the pathogenetic significance of solid aggregates in the blood stream of such different nature is obvious

In regard to Dr Knisely's sludges, he states (22) that there are "many different kinds of blood sludges" and the fact that he finds them in such a variety of clinical states as "hysteria" and "gonorrheal

test is made *in vivo.* There is fibrinolysin in the blood and in the tissues, and that can dissolve these things.

Steele: Well, these sludges do disappear right under your eyes; they move on and dissociate.

Heilbrunn: Oh, yes, there is this constant equilibrium between the factors that make for clotting and the factors that tend to prevent and reverse the clot.

Fremont-Smith: The statement that it was not fibrin, I think, is based on assumptions which are not altogether clear, namely, that fibrin doesn't dissolve again once it is formed within the blood vessel.

Heilbrunn: But it does.

Cowdry: It would be interesting for Gofman to discover whether there is any relationship between the sludges that Knisely has been studying and his macromolecules which he can separate out by centrifugation.

Steele: How would you go about dealing with these sludges of Knisely? You can't get them out of the vessels to study them.

Oliver: A good many years ago, we produced what would now be called "sludges," I think, by injecting arsphenamine into animals in large amounts (30). One could expose the mesentery of the animal, before death or after, and actually see the clumps with low magnification or the vessels could be cut and the blood allowed to flow out on a glass plate These clumps of red cells certainly were not "clots" in any real sense of the word There was no fibrin in them. The sludge or clump was made up only of agglutinated red blood cells that could be dispersed or brought together again *in vitro.*

Fremont-Smith: Wouldn't the statement that there was fibrin in them require further specification? That is, could one actually say that? It would take a chemical analysis or some physical analysis, wouldn't it, to prove it? It was the fact that sludges could be dispersed again which was accepted as proof of the absence of fibrin, wasn't it?

Oliver: Yes; immediately dispersed and immediately returned to an agglutinated clump at will.

Fremont-Smith: But if a little bit of fibrin were there to help to hold them together—

Oliver: Well, that would be something the pathologist doesn't call fibrin.

Fremont-Smith: That is the point I mean When is fibrin fibrin?

Oliver: There is a practical reason for distinguishing what the pathologist thinks of as "real" fibrin, because its physical state certainly cannot be reversed in any easy way or under any usual circumstances

Fremont-Smith: That is a very interesting thing. What we have now,

examined histologically the vessel of the brain, he thought he found tiny vessels that had been closed, with scars in the brain, and he believed that all of these accidents had been due to multiple closures of minute vessels. I think that is very comparable to what you may see in thrombosis.

Stern: To come back to cerebral arteriosclerosis and the things I mentioned before, it may interest you to know that in the brain we have a close parallel between symptomatologies when it comes to certain functions; for instance, pyramidal symptoms. If you have a case, for example, where you know that during life there has been a plantar extensor on one side, you can be sure that there is a lesion somewhere in the internal capsule on the opposite side. Now, when it comes to

described as certain lesions which he called "état criblé." These are lesions in which you have multiple small ischemic areas, for instance, in the pons, and characteristically, in the base of the pons. You have in here these small lesions. He found in many patients with arteriosclerotic Parkinsonism many small ischemic lesions with small, microscopically barely visible, holes in the basal ganglia Now, somebody has pointed out, and I have seen this, too, that people who have not shown any Parkinsonian features at all in life may have similar lesions; in other words, the parallel between arteriosclerotic lesions and symptomatology is not as mathematically accurate, you might say, as in pyramidal symptomatology The most striking thing of this type I have ever seen—and it is too bad that we don't actually publish negative cases—is the following. One of the typical lesions that has been studied for the

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on one side, you get hemichorea in the opposite side of the body, in arteriosclerotic individuals Lesions like that have been described by Vogt, by various French people, in the English literature, and so on I once saw a case of a woman whom I had known clinically literally to the last day of her life, and I happened to study her brain I did a post-mortem on her, and she had an old hemorrhagic cyst on the basis of arteriosclerosis in the head of one caudate Now, if that woman had happened to have hemichorea on the opposite side, I would have published her as a case report, because they are still comparatively interesting cases, but she had no extrapyramidal symptoms at all, in other words, you apparently need, apart from lesion and symptom, some third unknown factor, perhaps a constitutional predisposition or something like that

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All of these ideas are rather vague—a sensitiveness of the nervous system to lesions, something hereditary, I think, constitutionally. But if it comes to a pyramidal lesion, then I think there exists an absolute mathematical lesion-symptom relationship. I think that certain phenomena in the pathology of heart and kidney are analogous.

Steele: I would like to ask whether you think there is any way physiologically or histologically that temporary spasm can give you these vascular accidents or attacks or paresis.

Stern: Oh, yes. First of all, there have been true permanent hemiplegias, actual apoplectic stroke with permanent hemiparesis, and that has been described in the French literature. The French pathologists have cut the vessels in serial sections; for instance, let's say the middle cerebral artery and the branches of the corresponding area, and they could not find any occlusion In other words, they assumed that even a transient constriction or stasis or something like that might produce in some hypertensive individuals a permanent ischemic necrosis. For instance, patients who have multiple small ischemic areas in the brain, I think, often have in their history such things as a few seconds of blackout and dizziness and things like that, and these are probably subclinical apoplectiform insults There is one ophthalmologist who claims—I have forgotten the reference—to have watched the retinal arteries during one of those fleeting hemiplegic attacks, and there was an actual constriction, which means that there may be a constriction of brain arteries, too.

Fremont-Smith: Dr Richard Brickner has reported, I believe not too long ago, constriction of the retinal arteries, but this problem of constriction of cerebral arteries has been one that has plagued us, has it not, Dr Stern, for a long time, with great variation of opinion?

Stern: Oh, yes.

Fremont-Smith: And the best physiological experiments that I know of have failed to produce construction in cerebral arteries, with the exception of one—and that produces a general constriction—and that is overventilation or alkalosis In animals that are overventilated, where the CO_2 is blown off (and there is evidence in the human also of that), there is constriction but it is a general constriction that is not severe enough to produce definite ischemia (11)

Horvath: It is very minor, isn't it? It is a minor constriction The reduction in blood flow after hyperventilation has been very small (20)

Fremont-Smith: I think the reduction in blood flow is quite considerable, because the flow is so enormously influenced by a relatively small change in calibre of the arterioles. But you really know more about this than I do. I wish you would speak of it.

Horvath: My recollection of that is that the reduction in blood flow in hypertension is in the neighborhood of 10 to 15 per cent, in contrast to the tremendous increases that you can get, for instance, by carbon dioxide inhalation.

Fremont-Smith: By increasing CO_2?

Horvath: Yes. The reverse change is much, much greater. You can produce a greater amount of vasodilation or greater flow through the brain than you can obtain reduced flow. It has been very difficult, by any of the physiological alterations, to produce much of a change in flow without changing the general systematic flow at the same time. If you keep the general systematic level fairly constant, it is difficult to lower the flow through it again. Of course, gross techniques such as we have used only tell you part of the story. They don't tell you what happens in any one specific area, and therefore may not answer the question that you have raised, as to what happens in one particular area. You may have intense vasoconstriction in one area, because we do know some of those vessels under, say, local epinephrine or something like that, can constrict. Therefore, we can't answer the question. But the general picture is the small physiological change with relatively large increases of flow.

Fremont-Smith: For many years, it was believed by a good many people, and I myself included, that epileptic convulsions were precipitated by a vasoconstriction. Then the effort was actually made to demonstrate that, and I think the evidence now has proven that this is not so. I still like the idea that where there may have been an injury such as a post-traumatic head injury, those vessels may turn out to be more sensitive to circulating adrenalin, hypersensitive, as we know, in Raynaud's disease or other conditions where there has been a denervation, and there may possibly still be a basis for a temporary vasoconstriction in an injured area of the brain that could precipitate such an attack. But that is a longing for an old idea, and certainly the best evidence that Gibbs and others have is against that (12).

I think we are still not clear as to whether these transient losses of consciousness in elderly people, from which they recover without any residual clinical symptom, could be due to a constriction or whether they have some other mechanism.

I would like to go back to the question that you raised, sir, a few minutes ago, when you asked whether there was a relationship between the arteriosclerosis of the heart and heart function. I wonder if one could come closer to an answer to that by asking whether there is a relationship between the arteriosclerosis of the heart and the heart weight? — because increase in heart weight would certainly be an indi-

cation, would it not, of at least a compensatory need on the part of the heart. If one showed a correlation between arteriosclerosis of the heart and increase in heart weight, that would be an indication that the arteriosclerosis had, in fact, interfered with the function. Would you agree?

Oliver: I don't know.

Fremont-Smith: I mean, is that a reasonable approach to the problem?

Oliver: It is a reasonable assumption. I don't know as to the facts.

Steele: Wasn't there an old paper by Fahr (9) comparing hypertensive with nonhypertensive arteriosclerotic hearts? There was a very minor increase in weight in those hearts that had arteriosclerosis.

Fremont-Smith: Without hypertension?

Steele: Without hypertension, but it was very small compared to the ones with hypertension. It was just a 10 per cent, a marginal increase.

Fremont-Smith: It would seem, for one thing, that there certainly wouldn't be any one symptom correlation, because the position of arteriosclerosis on the coronary vessel will determine, as you pointed out, so much more the interference with functional capacity than just the degree of arteriosclerosis, which might be quite marked in certain places but never lead to disturbance in function because it didn't produce any occlusion

Steele: I think that is right, and the fact has always interested me I have been looking for a long time for somebody who actually has the signs of clinical, congestive heart failure, in an initial coronary occlusion. I have seen heart failure only when there seemed to be repetitive insults to the heart, with the electrocardiogram continuing to change over a period of six months or so. Also after two, three, or four coronary occlusions over several years, at some specific time, the heart may begin to fail.

Goldzieher: This question is a little more complicated than it seems, because if you equate heart size with arteriosclerosis, you omit another significant factor, namely the relation to heart size, irrespective of arteriosclerosis That brings us back to what Dr. Stern said and it seems to me a rather important point, namely that there is no correlation between arteriosclerotic encephalopathy and senile changes of the brain. The same holds true also in respect to other organs and tissues including the heart. Many years ago, I investigated the various tissues of aged people. In the course of these morphological studies, I was impressed by the observation that senile changes were particularly marked in individuals who had reached a ripe old age without much evidence of arteriosclerosis. This group included people who were

in their 90's and whose arteries would have been accepted gladly by most middle-aged people. Yet the heart of these oldsters was of exceptionally small size and showed marked pigmentation of perinuclear distribution similar to the brown pigment in the brain cells In other words, the morphological evidence clearly denoted senile atrophy which had developed in the absence of arterial changes. One might draw the conclusion from these observations that irrespective of the significance of arteriosclerosis in general, senescent changes do develop and reach an extreme degree without the contribution of arterial disease.

Shock: The phrase "cardiac function" has appeared a good many times in this discussion, but no one has defined what they mean by the term Do we mean "stroke volume," or the ability to increase cardiac output under increased demand? How do we judge "cardiac function?"

Steele: Well, of course, my judgment of heart function was a very simple and a very crude one — namely the occurrence of symptoms of insufficient cardiac output for whatever that person was trying to do. I think to go through the business of high output failure and low output failure is not worthwhile. Whether you happen to be hyperthyroid and need much more blood and your heart consequently fails to meet your needs with less damage to it than somebody who is not hyperthyroid, becomes of importance in knowing how great the damage is to the heart. But I know of no other way of distinguishing heart function except—

Shock: But don't we have any techniques for evaluating individual differences in cardiac function before frank failure ensues? Can't we detect early stages of impending failure?

Steele: That sounds like threatened pneumonia to me, but I don't know

[illegible]

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mi [illegible] strains of mice, where you could take a bit of cardiac tissue and, say, *put it in* some other place where it doesn't have to pump blood? What sort of aging changes go on in this tissue, apart from its cardiac function *in situ?* Has there been that sort of an approach to see what is going on in the tissue?

Steele: Well, you remember Dr Carrel's heart, that celebrated its birthday every year for a quarter of a century

Hamilton: Yes, but I am speaking of cardiac muscle Will the course of the transplant in inbred mice be able to be followed in a site apart from its thoracic location and function?

Cowdry: Wouldn't you expect that it would transplant perfectly easily, but not grow?

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Steele: Without hypertension, but it was very small compared to the ones with hypertension. It was just a 10 per cent, a marginal increase.

Fremont-Smith: It would seem, for one thing, that there certainly wouldn't be any one symptom correlation, because the position of arteriosclerosis on the coronary vessel will determine, as you pointed out, so much more the interference with functional capacity than just the degree of arteriosclerosis, which might be quite marked in certain places but never lead to disturbance in function because it didn't produce any occlusion.

Steele: I think that is right, and the fact has always interested me. I have been looking for a long time for somebody who actually has the signs of clinical, congestive heart failure, in an initial coronary occlusion. I have seen heart failure only when there seemed to be repetitive insults to the heart, with the electrocardiogram continuing to change over a period of six months or so. Also after two, three, or four coronary occlusions over several years, at some specific time, the heart may begin to fail.

Goldzieher: This question is a little more complicated than it seems, because if you equate heart size with arteriosclerosis, you omit another significant factor, namely the relation to heart size, irrespective of arteriosclerosis. That brings us back to what Dr. Stern said and it seems to me a rather important point, namely that there is no correlation between arteriosclerotic encephalopathy and senile changes of the brain. The same holds true also in respect to other organs and tissues including the heart. Many years ago, I investigated the various tissues of aged people. In the course of these morphological studies, I was impressed by the observation that senile changes were particularly marked in individuals who had reached a ripe old age without much evidence of arteriosclerosis. This group included people who were

Hamilton: I just throw it in as a possibility; getting a look at this tissue apart from its ordinary stresses.

Cowdry: It seems to me, Dr. Steele, that, when you talk about correlating the behavior of the heart with its structure, there has been so lamentably little done in the histochemistry and the straight chemistry of the myocardium, in the quantitative analysis of minerals and of amino acids, and in the distribution of enzymes, that there is not much to correlate

Steele: I guess you can count such studies on the fingers of one hand. I can think of only a few in and out of heart failure.

Cowdry: There have been some studies, of course, on the size of the cells and their nuclei but only by the simplest possible techniques. The more refined methods that have been applied to the nervous system, to the blood vessels and to the reproductive system just haven't been utilized

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Hamilton: But to be maintained.

Cowdry: Yes. However, there would be edges which would be subjected to phagocytic action and, gradually, the cells that are most prolific under those circumstances, the fibroblasts, would take charge of events and you would get a scar.

Hamilton: Has it been tried, say, in the anterior chamber of the eye?

Cowdry: No, this is pure prophecy.

Engle: Dr. Simms, have you had some experience with chick hearts along with your chick aortas?

Simms: No, only the embryo heart muscle grows.

Steele: Not adult?

Simms: No.

Engle: I think it would be completely fibrosed, just as Dr. Cowdry said. The cardiac muscle couldn't be maintained separately.

Steele: Of course, just recently, I think about a month ago, in *Science*, somebody cultured a chicken's heart and had beautiful growths of endothelial as well as connective-tissue cells. When he applied desoxycortisterone, he damaged the fibrous tissues much more than the endothelial cells

Hamilton: I know a whole hair follicle will continue to grow and produce structures

Cowdry: Are you going to take the myocardium of a very young animal that is still growing, or are you thinking of taking the myocardium of an adult animal?

Hamilton: I had in mind the embryo of an inbred strain of mice.

Cowdry: You would take embryonic tissue?

Hamilton: Yes.

Cowdry: Well, possibly you might, but I think the idea is that the myocardium of the adult does not grow It may increase in size, but the cells do not multiply

Hamilton: I was just wondering if there were some way to separate what is the result of changes in the heart as the result of its function *in situ* from what might go on if this tissue could be kept in a relatively—

Cowdry: Perhaps you wouldn't get the distinctive structural features of muscle to develop unless you had tension. I don't know; you might.

Fremont-Smith: That is a good point It probably wouldn't differentiate into muscle in the absence of a contracting opportunity.

Heilbrunn: The muscle tissue gets striation.

Cowdry: You get some striation in tissue culture but you are talking about taking muscle and transplanting it, which is a different proposition.

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find out how many people are interested in aging and in what aspects of it they are concerned. There had been too little emphasis, some of us felt, on the nonbiological and nonfinancial aspects of aging.

I say that, recognizing that last June, during the first meeting of the planning committee on the Research Section, I had to defend our decision to invite only forty or fifty biological research people out of a total of eight hundred, and I also say it, recognizing that the research group at the Conference felt that there has been very little research done on the aging processes. While that is undoubtedly true, a year ago not a single piece of research was under way on the topic of the relationship of private pension systems to retirement policies, which is certainly one, just one, of the very critical problems in this field. Also, to the best of my knowledge, there never had been up to that time any serious, concerted effort to do any research on the subject of living arrangements or housing for older people in this country. We felt that while there had been a good deal of progress made in the area of financing the later years and in the area of medical research, it was time to give people in the other areas an opportunity to catch up. So, we sought to stimulate more interest in those areas

We had no idea how many people would attend the Conference Actually, there were more than eight hundred After the Conference, there were quite a number of letters of commendation sent to Mr. Ewing That was probably to be expected, but some of those letters, I felt, really went out of their way and were more than mere gestures of recognition or gratitude for the Conference. The people who had attended conferences all their lives insisted that this was the best one they had ever attended

Now, I don't know why these people reacted that way. We have two hypotheses one is that it was the subject matter People actually were waiting for an opportunity to get together and discuss aging and its various ramifications The other, I think—and this may have been the more important one—was that this was a discussion conference.

We undertook to set up the Conference so that there were not more than twenty-five people in any of the discussion groups throughout the three days It was also a public forum, and many of the decisions were made by people outside the government who actually had responsibility for planning the content and techniques As a result some of the sections met in larger groups.

I have had a privilege that I think no one else has had, namely, that of reading the verbatim transcripts of all the sessions that were recorded Not all of them were recorded but generally the initial sessions and the final ones were, and some of the groups had records all the

SUMMARY OF ACTIVITIES OF FEDERAL AGENCIES

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Committee on Aging and Geriatrics

IN HIS opening remarks this morning, Dr. Fremont-Smith made some observations about the difficulty of finding a universe of discourse in which people from various disciplines could make themselves understood. As I have sat here today, I have not been able to observe any difficulty in communication among the participants with the exception of myself. I have appreciated the opportunity to be here today because part of my job, as I understand it, is to try to be somewhat *au courant* with what people in various fields are doing in the broad area of aging.

Dr. Fremont-Smith asked me if I would say something this evening about the National Conference on Aging and what has happened since that time, and what the Federal Security Agency's plans are. That I am very glad to do, although I warn you that I am going to toss the biggest immediate problems into your laps and seriously request your assistance.

In order to talk about the Conference, I would like to go back briefly to the time before it was held. I don't know whether or not you all know it, but the Agency is a kind of holding company for the Social Security Administration, which operates the financial programs that benefit the older people and other groups, the Office of Vocational Rehabilitation, the Public Health Service, the Office of Education, the Food and Drug Administration, and five or six smaller units

The Agency set up a Committee on Aging some time ago, and that committee met periodically I wasn't in the Agency then and I don't know how often it met, but I did get there at about the time the committee came up with the notion that the Agency was not concerned on a sufficiently broad basis with the problems arising from the aging of our population. It was particularly active in the field of financial aid, of course, and somewhat in the medical research field. But the committee became convinced, and the Administrator did also, that those problems represented only part of the total area in which aging was affecting our population and a good many of our institutions It was decided to hold a conference that would be broad in scope, partly to

Well, here are just some indications of the things that have taken place since the National Conference in August. On a national level, the Adult Education Association of the United States has now decided that one of the principal features of their annual conferences will be the subject of aging. The American Public Health Association had one paper last fall, I think, on aging. Next fall, they plan to have several sessions devoted to this subject. North Carolina called together all the persons from that state who participated in the Conference, and in May of this year will hold a statewide conference patterned pretty largely after the National Conference. They are spending a good deal of time, about seven or eight months, in preparation for it. There have been other states, such as Georgia, Michigan, Minnesota, and I think Dr Cowdry mentioned Nebraska this noon, where, not a comprehensive conference, but conferences that are statewide in nature are being called by educational agencies or welfare agencies or health agencies, and so on Some of them have been held and others are being held right along. Illinois appointed a State Commission on Aging just in time to be announced a day or two prior to the National Conference In Michigan, the governor has a committee, and the last I heard plans to appoint a statewide Commission on Aging. Just two weeks ago, the governor of one of our New England states called a number of us in for a discussion with some of the members of the Legislature, and he intends to ask the Legislature to set up a State Commission on Aging this spring

In communities, there have been similar activities In Philadelphia, there has been a Committee on Aging sponsored by the welfare and health organizations for a number of years, and that committee decided to get the conference delegates together—I think there were about twenty from Philadelphia—to do some further planning in the field. At the same time, the adult education groups in the city decided to do the same thing These two groups together drew up a five-year plan of objectives in the field of health, welfare, education, recreation, employment, and housing services, and they are asking the governor to set up a state commission Milwaukee is another place that has had a general meeting of people from a good many different fields, with a report on the Conference and discussion of problems in Milwaukee In St Petersburg, Florida, the health officer was stimulated to expand the services for older people Richmond, Virginia; Savannah, Georgia; Essex County, New Jersey, Denver, and Pittsburgh are other places where activities are going on. I might also include Kansas City.

There is no machinery actually set up for people to report what they are doing There is no effort on our part to get any systematic

way through. It is almost possible in these recordings to see thinking and development take place. I remember one instance particularly, where, in the Income Maintenance Section, there was a man who undertook to defend the retirement policy of his organization. He got up and said that when a man gets to be sixty-five they have a dinner for him and he has an opportunity to invite any of his friends that he wants to and they see him get the gold watch that they give him. He actually thought that that was something, until some of the other people began to tell about how that may be a shock to the individual from which he doesn't recover, or at least spends a long time recovering. They went to the other extreme of describing the functional retirement program; retirement based on capacity to perform, such as is being operated successfully in some places. Well, that, as I say, is merely one of the impressions that has come out of some of the correspondence that came in and some of the records that were left by the people who participated.

There is another kind of thing that I would like to talk a little about; that is, the reports that we have had of activities that have taken place around the country since the middle of August, and the requests that the Federal Security Agency has had from various kinds of organizations, communities, and so on. I certainly do not wish to give the impression that the National Conference on Aging last summer was responsible for all these activities. Some of them, I think, have existed for a long time and we are hearing about them now merely because the Federal Security Agency has established or announced its interest *in the field. I think some of these things were going on and, perhaps,* have been extended or stepped-up because as soon as there was national recognition or governmental recognition of the aging field, that constituted approval for some of the people who were more or less feeling their way along, with the result that they have gone ahead faster. I think also there have been some things that have actually been stimulated by the Conference

During the fall, I had occasion to make a summary of some of the correspondence we had had, in the Conference office alone When we made this summary (which was along in November, I think), we then had more than nine hundred letters. Now it is considerably more than that. Those represent letters that were properly handled in our office. I have no accurate count, but I know that there have been many, many additional letters, received by various units in the Public Health Service and Social Security Administration and other parts of the Agency and also other governmental departments, such as Agriculture, Labor, and so on.

whatever we do in the way of mobilization and increased use of manpower will be beneficial to our efforts in the aging field over a long time There may be a two-way relationship and recognition of it. It may also be that a good deal of interest in aging was generated and that mobilization hasn't hit us hard enough yet to cut off the interest in making more adequate opportunity or provision for older people.

I think it was Dr. Horvath who asked me today whether there was any tendency for people to think that with increased employment opportunities, there would be a tendency for people to say, well, their jobs are going to be available for old people and that will solve all their problems, and so we don't need anything else. That is a question that has been raised a time or two, but, interestingly, we have had only one letter on it

I am inclined to think that it is being increasingly recognized that the manpower reserve at the present time is not very large, certainly in terms of males It is nowhere near as large as it was at the beginning of World War II Nobody knows exactly how many older men there are who can be brought back into the labor force Certainly, the number of employed is almost at a long-time maximum right now, and we do know that if there are any significant numbers of males to be brought back in, it is going to mean rehabilitation programs for a good many of them We also know that, certainly, if there is a shooting war, we will not have sufficient manpower without drawing on the ten or twelve or more million women who have passed middle age, and that to draw them into the labor force is certainly going to call for more than just opening jobs Many of those women never have worked and others haven't worked for twenty years or so, and it is going to be necessary not only to change their attitude toward work outside the home but also to provide training for them.

Well, that is one of the questions that is giving us some concern, and no one knows yet how many of the people are going to have to be brought into the labor force, or when or where. The Agency is undertaking to define its role in the field.

The section in which most of you who are here were present last summer recommended that the Agency set up an advisory committee on gerontology. There were four or five other sections that recommended that the Agency should set up a unit which would serve as a clearing-house of information in the whole aging field and perform certain other functions There were, on the other hand, some groups that felt

reporting, but at the time I made this tabulation, we did list a number of states from which we had had indications of activity. At that time, there were twenty-four. I assume that there are more that have not reported to us.

Those are state and city activities. I have been very much interested to see in magazines of the Presbyterian and Lutheran churches, i.e., of their national organizations, that they are now recommending to their constituents that old-age homes should not be located out in the country but within the community, where the residents can maintain contacts with their friends and relatives and the institutions in which they have spent a good deal of their time. It may or may not be an outgrowth of the Conference, but those people were present and that is one of the points that was recognized there.

The General Federation of Women's Clubs of the United States has set up a Division on Aging, and Dr. McCay was telling me today that the New York State branch has similarly set up a division I am not going to be here tomorrow because the Tacoma Park, Maryland, branch of the General Federation is having a meeting on aging, so it is seeping down through their organization into their smallest units.

There have been several new research activities announced during the summer and fall The Twentieth Century Fund, as some of you probably know, has two outstanding economists working on the question of private pension systems. The Rockefeller Foundation is supporting the University of Chicago in a study of the retirement practices of industry and there is an investigator making the rounds. I haven't seen the schedule that she is using but she read it to me over the 'phone the other day, and if industry co-operates, she will have a tremendous amount of information regarding the attitudes and practices of industry with regard to retirement of older workers

The University of Florida, according to the last I heard, is still organizing a gerontological research conference to be held sometime before next summer *

Well, that is just a sample of the kind of thing that is going on around the country at the present time So far as I know, I think every single field of activity is represented and active Now, one of the questions that concerns us is why has this interest expanded, or, if not expanded at least persisted, during a period when we are supposed to be thinking about mobilization for total defense? It is possible, of course, that people are tying the two together and recognizing, as the Department of Labor and the Federal Security Agency are increasingly

*Editor's note This conference was held at the University of Florida, Gainesville, on March 19-20, 1951

I know very well that, while there is much talk of the desirability and of the essentiality of setting up interdisciplinary or interagency groups, there are few successful examples But once in a while there does occur a demonstration of people from various disciplines, or of several organizations getting together and working together, and we are going to try it. The Committee on Aging and Geriatrics will be essentially a working committee meeting fairly frequently and exchanging information and ideas, much the way you have done it around this table. The representatives will carry back to their own organizations some of the ideas developed in the meetings, thus, we hope, stimulating new activity.

Then, we were thinking of an advisory committee, which is the kind of thing, I believe, that was recommended by several groups at the Conference—an advisory commitee of nongovernment people. I think the general practice in Washington is that a committee of this kind usually has somewhere from ten or a dozen up to twenty or perhaps twenty-five members That is about the number that can be managed. It is not very many members, however, for a committee in the field of aging, since aging covers so many aspects of life. That is one reason why our thinking is directing us toward a larger group. Another is that we discovered, through the Conference, that by involving a great many people in it, we stimulated a good deal of interest around the country About two hundred nongovernment people participated in the Conference and probably made an investment of interest that has a carry-over value Our thought is that if we can set up an advisory committee rather large in terms of numbers, we may help to sustain the interest in aging among more people and across a larger part of the country

What we are thinking about, therefore, is a committee that might have fifty members or sixty or perhaps even a hundred or more, which committee probably would never meet as a total group but would be consulted by mail, as the advisory committee for the National Conference was consulted, and from which it would be possible to bring in small, working groups on a particular problem at a particular time As I say, this advisory committee idea is in the thinking stage, and we would certainly like to have your views on it, as well as your advice on the kind of things the interdepartmental working Committee on Aging and Geriatrics should undertake to do.

We do not view ourselves as a major operating agency in this field. Our feeling is that most of the work in this area is going to be done in local communities and institutions and organizations, throughout

then, and still feel, that the Agency should not extend its interest beyond that which is already represented by the Public Health Service and the Social Security Administration. However, the communications that I mentioned a few minutes ago were not sent to us merely to tell us what is going on. They were sent because people wanted help on planning conferences. They wanted the names of speakers who could be brought into their conferences. They wanted suggestions on how to organize local groups, where to build old age homes, what kinds of programs to set up in them, how to set up recreation centers and educational programs, and so on. Some wanted bibliographical material. For three months, I have been referring them to the comprehensive bibliography Dr. Shock has prepared (1).

They want information on standards for building homes for older people, and statistics on various aspects of the problem, and so on. Therefore, actually, we have been serving in a very small way as a clearinghouse of information in this field, and the announcement of the appointment of a Committee on Aging and Geriatrics, was the first step in trying to provide a clearinghouse for information in a more systematic way than we have been able to do it so far. The Committee will provide, we hope, some consultation service of a technical nature, largely to states and communities and organizations that request it.

Another function that I neglected to mention was that we have had quite a number of requests from newspapers, magazines, radio stations, and now television for material that can be used in articles or programs, and we undertake to cooperate with them because we feel it is important to get more and more people thinking in this area

What other things we will be able to do, I don't know, and when we will be able to do them, I don't know I think that the announcement that the committee has been set up will probably have a little bit of the same value that the Conference did, that is, the mere knowledge that the Federal Government has recognized the importance of aging will provide encouragement for others to go ahead with their own programs.

Actually, the committee is just now being set up We are calling together a group of people at the operating or working level in those federal agencies which have some direct concern at the present time with the problems of aging. It will include the several units of the Federal Security Agency, several of the units (three or four, I believe) in the Department of Labor and in the Department of Agriculture, as well as representatives of the Federal Housing and Home Finance Agency, and perhaps the Veterans Administration, and the Census Bureau. It will be an interdepartmental committee.

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We do not view ourselves as a major operating agency in this field Our feeling is that most of the work in this area is going to be done in local communities and institutions and organizations, throughout

the country and that our function will be largely one of helping to spread information and helping to expedite matters for others.

Now, just one more word, and then I will stop. We are concerned that interest in this subject should hold up, and for that reason we are very happy to know that Dr. Cowdry and his group have planned to go on with the Second International Gerontological Congress despite the fact that there may be difficulties—perhaps there are already difficulties—in making it broadly international. Those of you who were present will recall that at the final session, Mr. Ewing urged Dr. Cowdry to go ahead, and promised help. I am glad to say that we have been able to be of some little assistance in some aspects of the planning for that conference. I think Dr. Cowdry plans to say something more about it now.

DISCUSSION

Cowdry: I have been interested in aging for a long time, and it is very nice to see the Federal Government coming into it in such a comprehensive, well-planned, and far-seeing fashion. I think it must be gratifying to the officers of the Macy Foundation especially because you will remember that the Unit on Gerontology of the Public Health Service was almost unique in that it was started by co-operation between the Macy Foundation and the United States Public Health Service back in 1940. The Macy Foundation, as I recall, paid the salaries—is that right?

Frank: Yes. The Macy Foundation gave the money for the Unit on Gerontology in the Public Health Service for the first year, and also for the meetings of the advisory committees

Cowdry: Well, my memory is not so bad as it might be, then. It is very pleasing, I am sure, to the Macy Foundation—and I am sorry Dr. Fremont-Smith isn't here to listen to this statement of how well it is turning out. We are seeing a kindling of interest pretty much throughout the country in the subject of aging It is different in a sense from the kindling of interest in cancer, but it is, I think, almost as widespread since it affects so many different groups. There are probably as many metings on aging at the present time as there are on cancer I want to congratulate Dr Tibbitts on making such a good presentation without the use of the word, "gerontology," which always sticks in my crop. I think it is one of our handicaps. However, we've got it and we have to use it for our international congress, because it was wished on us. Even the book that we published on aging some years

;o may take credit because "ageing" was spelt properly and because e didn't use the word, "gerontology."

Frank: We dropped the "e" this time, Dr. Cowdry.

Cowdry: Yes, I noticed that. Anyway, we are friends together and little self-criticism isn't a bad thing at times.

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FORECAST OF THE SECOND INTERNATIONAL CONGRESS ON GERONTOLOGY

EDMUND V. COWDRY
Department of Anatomy
Washington University School of Medicine

BUT NOW a word about this congress, or several words.

This congress is the result of a meeting at Liége last summer (1, 2) at which the Gerontological Societies of about a dozen different countries got together and recommended that a Gerontological Congress be held in this country in September of this year (3).

The organization of this congress has not been easy. It has been difficult for several reasons One of them is the failure of anybody, or most people, to see anything in the nature of an emergency about aging. Aged people are, however, under a sentence of death which is more immediate than that of young people. It is just as definite as the sentence of death from cancer and there is no avoiding it, whereas most of us manage to escape getting cancer.

Our problem in this congress is mainly and almost entirely to assist foreign governments and individuals to attend, that is, to make it international. To that end, we have adopted a number of devices to raise money for the congress

First, we wrote to all foundations in this country, with the exception of the Ford Foundation which we are reserving for a more direct and special approach. All of these foundations, except the Macy Foundation, *declared in writing that it was against their policy to foster international congresses.*

The Macy Foundation has generously contributed $5,000.00 to the congress. The Public Health Service first turned down our request for aid and then suggested that we make a second request, which has been done, for $10,000.00 * Coupled with this $10,000.00 request is the statement from Washington University that it will duplicate the Government's grant. We are attempting to raise money in St. Louis, and had a meeting recently. I was informed by Mr Howard Young, who is the president of the largest lead company in the country, that

*On recommendation of the National Advisory Heart Council this grant has been activated by the Division of Research Grants and Fellowships, National Institutes of Health.

he thought we would be able to raise $10,000.00. We have promised Dr. Brull, who is president of the international organization, that Washington University, from its $10,000 00 grant, will pay to as many as twenty people attending the congress, $500 00 for their expenses in this country on their arrival in New York. We hope that this will encourage them to use their own funds, in their own currencies, to purchase trans-Atlantic travel. We think it will.

Lord Nuffield has promised £750 It is possible we may get more. The *Journal of Gerontology* is saving us a lot of money by agreeing to print the program containing abstracts of all the papers and the descriptions of the exhibits as a special supplementary issue of the *Journal*, which will be distributed to all those registering at the congress. The registration fee will be ten dollars. Of that, we shall pay the *Journal of Gerontology* only a dollar a copy for these programs. I recall that the program for the cancer congress cost us about $6,000 00. One thousand copies of the gerontology program, despite the increase in cost of materials and labor, of about the same size, will cost us only a thousand dollars, which is a great saving.

We think that we are going to be able to get good foreign representation The people abroad have sent us a fine list of papers, which they would like to present if they can be helped to defray the costs of travel We aren't saying who comes on their money or who comes on our money That is for them to say We believe it is better for us not to try to pick and choose We shall follow closely Dr Brull's recommendations

A series of exhibits is being planned which we think is going to be worthwhile The chairman of the Committee on Exhibits is Dr. Albert I Lansing He approached certain industrial firms and has thus far received a thousand dollars and the promise of another thousand We do expect to get more

The two great issues, in addition to financing, are, first, can we get the State Department to help us with this congress in the same fashion that it helped the cancer congress some years ago? We think that we can You may recall that for the cancer congress, the State Department, thanks to the kindness of Dr Scheele, transmitted our invitations to all foreign governments to attend the congress This made a great deal of difference especially in securing co-operation from South America. Altogether forty-four nations were represented. In addition, the State Department, at the conclusion of the cancer congress [illegible] summary of the results [illegible]
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[illegible]

[illegible] the people at home

they did a good job. We want to get similar help from the State Department now with this International Gerontological Congress at the earliest possible moment, because time is short.

What the State Department has done thus far is to say that it will transmit the names of all those planning to attend the congress to the heads of American missions abroad, so that the obtaining of visas may be facilitated. This is a very different thing from calling attention of foreign governments to the fact that the congress will be held and that they will be most welcome and that we want to have them officially represented.

To help us is in direct line with the policy of the State Department, which is to strengthen international relations by all possible means, despite the clouds that may appear on the horizon.

Another ambition is that this congress in St. Louis shall be a direct extension on an international basis, of the National Conference on Aging so ably directed by Mr. Tibbitts in Washington last August, and I am making every effort to induce the powers that be to permit Mr. Tibbitts to be the official director of this congress in St. Louis. I think it is very important that he should be.* We want to grow from the strength already established and become stronger through use, and we want to receive these people from abroad into a development already well under way, thanks to the National Conference in Washington.

I have told you our ambitions, our aims, and our difficulties. We are trying to carry on. We want help. Just now, there is a kind of a plan maturing for some of these people attending the congress from abroad to make a tour through this country, to visit Canada, the Pacific Coast, come back through Mexico City, and out again through New York—perhaps half a dozen of them. When we can make a list of first-rate scientists who are good speakers and have messages to deliver of interest to university and other circles, we propose to circulate this list in an effort to provide a sort of touring set of minstrels to toot the importance of the problems of aging. Stock in the importance of the problems of aging will go up and there our welcome visitors will, we trust, receive generous honoraria which will take the edge off the cost of travelling so far.

We are now trying to get people interested and to appoint a committee which will make the arrangements.† These arrangements would have to be binding because those coming over would count on a cer-

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*Mr. Tibbitts was subsequently appointed Vice President of the congress.

†A committee to help arrange lecture engagements for those attending the congress from abroad was organized in March, 1951. O. J. Kaplan, chairman, C. D. Leake, E. Moore and F. Hinman, members.

tain amount of revenue from this tour, and it would be most embarrassing if they came over and the revenue did not materialize.

We are helped by action of the Dutch Airlines in providing very cheap transportation It amounts, I think, to $400.00 for the round trip. The only difficulty is that travel to the United States must be in July and the return in September which leaves the individuals a little longer here than is necessary.

Another task is to obtain for our visitors possible Fulbright grants Thus Professor Albertini, from Zurich, who would like to attend this congress, has been invited by Washington University to serve as visiting professor of pathology for three months Though Switzerland is not a Fulbright country we expect to get help for his travel. We are attempting to secure Fulbright grants and accompanying university appointments in the United States for several others We must pass up no opportunity, however small, to give assistance to our foreign colleagues so that they may attend the congress

DISCUSSION

Frank· Mr Tibbitts raised a question about an advisory committee and the working committee and what they do and what we could suggest as worth their consideration, and then what suggestions you might have about the international congress. One point I would like to lead off with is this: There is going to be increasing need, not only for a clearinghouse to answer inquiries, but also some sort of an arrangement whereby you can send out competent consultants to communities and states Within the various agencies of the Public Health and other agencies, you have people who could be very helpful to communities if they could actually go out and advise them. I should think these consultants would be very useful, because there is nothing like having somebody who can go and sit down with a group that is planning a medical care program or public health care or day center care for older people

Tibbitts The principal problem there is to get the various units to recognize that this is a field to which they should permit their staff members to devote time I am hoping that that will proceed rather rapidly.

Frank· Another point would be to try to get up a panel of possible consultants who are not in government services, by regions, who could be called on for advisory services. The Gerontological Society could possibly do something to help to build up such a panel from its own membership

Tibbitts: In a case like that, who would compensate those people for their expenses?

Frank: The organizations using their services would have to pay them directly. It could be organized possibly, through the Gerontological Society, as a way of providing that kind of centralized service, with standard fees, plus traveling expenses.

Tibbitts: What, specifically, about this question of a small group that could be brought together periodically, versus a really good-sized one that would probably never meet?

Cowdry: To have a group of advisers sufficient for your conference, or as we have for the congress, would be the best way. We call on these advisers repeatedly for suggestions, and get very good ones. They feel active, which is a good thing. If you call the advisers a "committee," you almost have to invite them to attend meetings, which is not necessary if they remain a group of advisers at your disposal individually

Frank: There could be a panel of advisers from whom you can call, as you say, special small groups. They could be utilized when you have specific things you want to do One other aspect of this I am concerned about, namely, that different communities and states are going to feel the pressure for very specific programs in their areas. That is where the new committee could be useful, by pointing out that no plan for the aged can be done effectively in isolation, without realizing its implications and the need for the various other services and facilities. That may prevent these one-sided programs that may be attempted, with just a medical program or just a financial program without the necessary concomitants More and more we are realizing that study and care of the aged is a multiprofessional, multidiscipline job Somebody will have to keep repeating that and keep calling their attention to it; otherwise they will forget about it

A number of communities, such as Michigan, Chicago, and California, have established seminar programs for the coming summer. Perhaps some of the visitors to the International Congress could be used in these programs.

Cowdry: We expect that the University of Chicago Round Table radio broadcast on the Sunday before the congress will provide a good start We greatly need advice on publicity beginning soon. Are there any suggestions?

McCay: I should think the dates for the congress would be very good for summer schools All we need at our school is about two months' advance notice to apply for lecturers July is very good, because the summer schools are opening about July 1

I wonder if you have tried Anheuser-Busch or Borden for support. Both of them have some interest in the subject of aging.

Coudry: We aren't forgetting them.

McCay: I hope not. I also wonder about Borden. They gave a $1,000 00 award for research on old people to Miss Olsen of Michigan State College last year, you know. As I think about the breadth of your committees, we have several operating, you know. We have Senator Desmond's committee in New York State, and now we have a new advisory committee for the Federated Women's Clubs. We think aid for the aged hasn't been sufficient in the past; that there is need for much better legal advice for older people, and certainly for advice on their investments and securities. We have also advised the women's clubs on the need for reasonable facilities in libraries of various sizes in our state We have suggested that they set up lists, varying with the population, and then see that their libraries have a reasonable literature in this field. I think, on any committee like that, we need good advice on libraries, and libraries of various sizes, as an initial step to the Federated Women's Clubs in our state And then, in their operations, I should think, again on committee cooperation, we might suggest that now, if ever, there is great need for improved basic foods, because of the inflation that is certainly catching the old folks. You can see it in New York You see the old folks eating mostly carbohydrates in the restaurants. We suggest that they ally themselves with the Home Bureau operations on better food for the older people in the community, and we also try to get them to ally with the State Nutrition Committee

There is certainly much need for a co-operating housing service. We have tried three [illegible]

time [illegible]

[illegible] at all We don't know what

[illegible] field generally, there is much need for reviewing food allowances right now I am working in our local area. Our food allowances are based on composite prices, which go into the state and then are returned to us as allowances At present, for old people, the maximum is $24 00 per person per month. We are beginning to wonder whether this isn't too low.

There is certainly much need for consideration of church interests for older people I am a Unitarian and we are holding a roundtable discussion next week on the general problems of aging and what the church can do to improve the situation The other night we held a seminar meeting at the Torch Club in Elmira, which, as you know, is

a businessmen's club That was the second meeting they had concerning aging. I am just thinking of the interests that are arising that we are little aware of, but we do need stimulation from some central group such as you are developing

Tibbitts: That is one of the things we want—what kinds of interests and what kinds of people should be on such committees?

Hamilton: Dr. Cowdry, when one thinks of aging, one sometimes thinks of cosmetic and similar concerns. I was charged with raising money for the hair conference the New York Academy of Science held last winter, and we found various concerns like Lever Brothers and others more than receptive to helping support the conference costs. I think aging might have been a better appeal to many business concerns.

Cowdry: Where would you direct a request to Lever Brothers? Chicago?

Hamilton: Amos Light of Burroughs-Wellcome can give you a list of those who contributed to the hair conference

Shock: There is another possibility for obtaining financial support—from the drug and pharmaceutical manufacturers

Horvath: Have you tried the fraternal organizations like the Moose, the Elks, or the Masons? Perhaps business clubs, like the Rotary or Kiwanis, would help out.

Cowdry: The St. Louis Kiwanis will contribute $50.00 to a gerontologist attending the congress who will give them a lecture.

Frank: Is there any place where we can find out the practices, the conditions, the charters, the arrangements under which they are operating homes for the aged, such as the Elks, the Actors, the Moose, and so on?

Shock: The Department of Labor has put out two publications, with a detailed description of the admission policies and so on, for all homes for the aged in the United States (4)

Hamilton: May I ask if your visiting scientists would be available when school is in session? You spoke of the period from July to September.

Cowdry: They could be

Hamilton: The reason I ask is that in our school, there are various arrangements for visiting professors who can speak English and who are of the caliber that the physiologist, say, would like to have in physiology.

Cowdry: One of the people is a codiscoverer of vitamin K, a Nobel Prize winner, who is most eager to come. It ought to be possible to get him in somewhere.

Hamilton: If you had a list of those people, I am sure if we brought

it up to the Committee of Administration, we could see which names on the list would interest the various people who control these visiting professorships. But they would have to go during the school year rather than a three months' period.

Cowdry: Well, they could come at the beginning of the conference and then extend their stay.

I see lots of work ahead of me.

Frank: I think we ought to say that if you have any further suggestions, send them to Mr. Tibbitts by mail or otherwise, and he will be very glad to have them.

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AGING OF THE INTEGUMENTARY SYSTEM

EDMUND V. COWDRY
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Washington University School of Medicine

I AM GOING to try to present the subject of "Aging of the Integumentary System," as it has been phrased.

First, it is desirable to acquire an appreciation of the conditions of cell life in the epidermis. I believe it is fundamental to distinguish chemically between the epidermis and the dermis beneath, and so a method was devised whereby we can loosen the epidermis from the dermis merely by slight heat, and strip it off and study it chemically by analysis or microscopically as an entire sheet of tissue. Microscopic observation shows an irregular line of empty-looking spaces where the grip of the dermis on the epidermis has been relaxed by liquefaction of the collagenic fibers (1)

It is important to get an idea of how very different the chemical composition of epidermis is from that of other better known tissues. I would draw attention especially to the difference in calcium content (Table I). There is actually more calcium per gram of epidermis than for any other tissue in the body except bone and teeth Another component present in enormous amounts is urea.

McCay: Is this human skin, Dr. Cowdry?

Cowdry: No, this is mouse skin.

Oliver: Are there species differences?

Cowdry: The species differences are very slight We haven't been able to make as complete analyses of human skin because we haven't been able to get sufficient samples in suitable condition, but those we have made approximate very closely the analyses of mouse skin Table I was prepared by Dr. Carruthers and published in my textbook (10).

Shock: Can you get enough skin from a biopsy specimen for analysis, or do you need larger samples?

Cowdry: No, you cannot You have to get more than a single biopsy. You usually get human specimens at surgical operations. If the operation involves a laparotomy, you can usually get a good deal, because the edges of the long incision are drawn together so that the person can't tell when he has been robbed You obtain most skin from amputations, of course; but its normality is questionable

TABLE I

Chemical Composition of Epidermis Compared with Liver and Muscle

	Epidermis	Liver	Muscle
Cytochrome oxidase Qo_2[1]	24 9	392.0 r	180 0 r
Succinic dehydrogenase Qo_2[1]	3 7	87.7 r	36 0 r
Apyrase[2]	3 0	12.9 r	23 3 r
Arginase[3]	32 0	2453 0 m	—
Cytochrome c—μg/g w w.[4]	52 0	90 0 r	97.0 r
Urea—μg/g w w	770 0	300 0 r	—
Ca (μg/g w w)	440 0	90 0 r	100 0 h
Mg (μg/g w w)	19 0	190 0 h	270 0 h
Na (μg/g w w)	168 0	305 0 h	72 0 h
K (μg/g w w)	347 0	72 0 h	365 0 h
Ascorbic acid—μg/g w w	240 0	320 0 m	43 0 m
Biotin (μg/g dry weight)	0 196	1 87 m	0 086 r
Choline (μg/g dry weight)	2471 0	5280 0 r	—
Inositol (μg/g dry weight)	526 0	933 0 m	466 0 r
Pyridoxine (μg/g dry weight)	2 45	4 66 m	3 03 r

1 Qo_2—oxygen uptake per mg dry wt. per hr

2 μg P liberated under standard assay conditions.

3 μg urea liberated under standard assay conditions

4 w w —wet weight of tissue

All epidermis analyzed is from mice The sources of the liver and muscle are indicated by the letters h—human, m—mouse, r—rat Table prepared by Dr C Carruthers

Reprinted from—Cowdry, E V *A textbook of histology* Lea & Febiger, Philadelphia, 1950 (p 551)

It is important to realize that no matter what you do, there is going to be considera

The shrinkage

Engle: Is that merely loss of water, Dr. Cowdry?

Cowdry: No, I don't think it is I think it is partly due to the fact that the young skin is highly elastic and the old skin is less elastic. You know that old trick of placing the hand relaxed and palm down on the table and telling the age of the person by pulling up the skin and noting how it subsides when released When you do that with a senile person like me, it sinks back slowly and with dignity, but if you take a young person and put his hand on the table and pull the skin, it snaps back with the speed of youth.

Engle: Well, Dr Cowdry, this is surface area then, and there would be a thickening of the skin as it shrank in so far as it wasn't water loss, isn't that right?

Cowdry: Yes, that's right.

Engle: And the loss of weight would be a water loss primarily?

Cowdry: Yes.

Fremont-Smith: Is there a difference in the loss of weight in the young and the old?

Cowdry: I think there is some.

Hamilton: Is there any direct evidence of actual differences in elastic tissue in young and old skin?

Cowdry: Yes, there is. That is what I was going to discuss later, but there is no reason why we shouldn't discuss it now.

Hamilton: I withdraw it until later.

Cowdry: We are trying to discuss first the aging of epidermis and then that of dermis.

The thickness of young and old epidermis is not very different, a mean of 33.8 microns for the former and 27.3 microns for the latter. The reason why there isn't a great difference in thickness is that the specimens were taken from the antecubital space where age changes are not very conspicuous because the region is protected. All samples of skin show to some extent layering of epidermis as well as the connective-tissue fibers that constitute the basement membrane. There is a concentration of blood vessels in little projections upward from the dermis. Skin samples from the sole of the foot show maximal layering, and a typical absence of the granular layer and a tendency to split along definite layers.

The cells of the follicle show a negative Schiff reaction, while the cells of the epidermis, continuous with the outside, show a positive reaction.

One of our graduate students in St Louis has found it possible to remove the granules from the granular layer of the skin, collect them *en masse*, and study them chemically. This has not been published, of course. This is an example of methods whereby we can collect parts of the epidermis in quantities sufficiently large for chemical analysis.

Whole nuclei of epidermis can likewise be collected *en masse*. This is more of an achievement than the separation of the nuclei of liver cells because the epidermal cells are so much tougher and are more tightly bound together. It was first done by Dorothy Ziegler Kramer in our laboratory (31)

You can go further in fractionating parts of the skin and take out the chromosome threads from the epidermis They make a mass of surprisingly uniform individual threads when observed microscopically Such masses have been analyzed by Gopal-Ayengar and by me (19). This, like the collection of keratohyaline granules and of whole nuclei, was made possible by employing the technique of differential centrifu-

gation It is also a relatively simple task to remove the mitochondria from epidermis and to analyze them, too.

Now, in continuation of this examination of the problems involved in the investigation of aging epidermis, I want to emphasize regional differences Table II lists some of the regional differences in the primary location in the epidermis of various lesions The primary localization of a good many of them is fairly sharp and not always easily explained. Consider, for instance, the first appearance of vitamin A deficiency lesions on the extensor surfaces of arms and legs and of riboflavin deficiency lesions on the sides of the nose, oral angles and canthi

Sheets of whole epidermis may be separated by dilute acetic acid and prepared for microscopic examination by simply staining with hematoxylin, placing the inner side uppermost, and mounting in balsam Skin taken from the neck (suprasternal notch) shows clearly the ducts of some sweat glands and a piece of a hair follicle with attached sebaceous gland When you compare the epidermis of the newborn and the eighty-year-old man, there are profound differences, which are presented briefly as a basis for discussion The first thing you notice is that the ridge-like thickenings of epidermis appear to be ironed out in the older person, and the sweat glands are very greatly reduced in

TABLE II

Primary Location of Skin Lesions

Lesion	Primary Location
Vitamin A deficiency	Extensor surface of the arms and legs
Vitamin B (riboflavine) deficiency	Sides of nose, oral angles, canthi
Hyperhidrosis	Palms and soles.
Acrochordon (cutaneous tags)	Neck, axilla, upper parts of the thorax
Amyloidosis cutis	Anterolateral surface of the legs
Senile angioma	Anterior chest and abdomen
Neurodermatitis	Antecubital and popliteal areas and sides of neck
Erythema multiforme and icthyosis	Extensor surface of the arm and forearm
Acanthosis nigricans	Mouth, axilla, groin, navel.
Psoriasis	Scalp, elbows, knees
Acne	Face

Reprinted from—Cowdry, E V, Cooper, Z K., and Smith, W Program of research on ageing of the skin J Geront, 2: 31-44, 1947.

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Hamilton: I withdraw it until later.

Cowdry: We are trying to discuss first the aging of epidermis and then that of dermis.

The thickness of young and old epidermis is not very different, a mean of 33.8 microns for the former and 27.3 microns for the latter. The reason why there isn't a great difference in thickness is that the specimens were taken from the antecubital space where age changes are not very conspicuous because the region is protected. All samples of skin show to some extent layering of epidermis as well as the connective-tissue fibers that constitute the basement membrane. There is a concentration of blood vessels in little projections upward from the dermis. Skin samples from the sole of the foot show maximal layering, and a typical absence of the granular layer and a tendency to split along definite layers.

The cells of the follicle show a negative Schiff reaction, while the cells of the epidermis, continuous with the outside, show a positive reaction.

One of our graduate students in St Louis has found it possible to remove the granules from the granular layer of the skin, collect them *en masse*, and study them chemically. This has not been published, of course. This is an example of methods whereby we can collect parts of the epidermis in quantities sufficiently large for chemical analysis

Whole nuclei of epidermis can likewise be collected *en masse*. This is more of an achievement than the separation of the nuclei of liver cells because the epidermal cells are so much tougher and are more tightly bound together. It was first done by Dorothy Ziegler Kramer in our laboratory (31).

You can go further in fractionating parts of the skin and take out the chromosome threads from the epidermis They make a mass of surprisingly uniform individual threads when observed microscopically Such masses have been analyzed by Gopal-Ayengar and by me (19). This, like the collection of keratohyaline granules and of whole nuclei, was made possible by employing the technique of differential centrifu-

as senile keratotic areas. In senile keratotic lesions the decrease in the mineral residue is mainly in calcium and/or magnesium. It is more [illegible]

mineral

In any study of epidermis one should realize that an increase in number of cells by itself does not tell us very much. We remain ignorant as to whether the increase in number of cells is due to an increase in rate of mitotic division, to longer life span, or to decreased death rate In such a large cellular population it is always possible that a few individual cells may exist in conditions of life entirely different from those of the vast majority

Horvath. Dr. Cowdry, may I ask you a question?

Cowdry: I want you to.

Horvath These changes in epidermis that you are showing here are primarily of the face, which is an exposed area Do you see similar changes, say, in the back or the abdomen with age — referring now simply to age?

Cowdry. You see changes there, too, but they are less marked. For example, in the skin of the back and of the abdomen, it is easy to detect a decrease in the number of sweat glands with age. The exposure to the environment, of course, is comparatively slight, and the aging in such protected areas is not so marked Our study did include these other regions, too

Horvath: I was just wondering whether these were just a little more marked

Cowdry: In [illegible]
course, in c[illegible]
spreads fro[illegible] to others, as in *xeroderma pigmentosum.*

Letm: In your analysis of the mineral content of skin, you pointed out the large increase in calcium I was wondering if there is any difference in calcium content in the various layers of the epidermis as you go from the inside out My feeling is that as you progress outward and encounter increasing *keratinization*, and I would also expect increasing dehydration, that possibly the minerals would be more concentrated in the very outside layers of keratinized cells, as compared to the inner layers, which are presumably more viable, less keratinized and less dehydrated

Cowdry: You are perfectly correct in your thought that there will be marked differences in the amounts of mineral in the different layers

number The clarity of the background is noticeable in the young as contrasted with the density in the older person. In the latter the glands and hairs are greatly reduced in number. Different regions of the body exhibit the features of aging rather differently. Dr. Zola Cooper, Dr. W Smith, and I have studied them in cooperation (11).

Oliver: What is that clarity or the lack of clarity due to?

Cowdry: I think the clarity in the very young or the newborn is partly due to thinness; but there is more to it than that.

Sheets of epidermis from the forehead do not show quite the same age differences. Some hairs may still be seen projecting out of the follicles, though many are lost in the old man.

Comparisons of the epidermis from the temple of the full-term newborn and the eighty-year-old man are interesting Skin from the old man shows thickening of epidermis and a curious pattern of distribution. The background seems to be bleached out in some cases and doesn't have the uniformity that is evident in the newborn.

Nelson: What does that pattern result from?

Cowdry. It is due to localized thickenings of the epidermis, a kind of hyperplasia. Precancerous epidermal lesions are fairly common in this area of skin of old people. The earliest ones should be studied in whole mounts like these.

Before I go on, I would like to summarize for you what, it seems to us, is important about the aging of the epidermis, as you see it microscopically. There are many instances of processes which seem to balance yet are subject to displacements in one direction or in the other. There is, for example, atrophy and hyperplasia in the epidermis There are areas which are thinner, and there are areas also which are thicker, as you know These can be sharply circumscribed or they can be rather diffuse. There are areas in which there is hyperpigmentation and hypopigmentation. We see this in all old people The hairs may drop out and disappear, which is a common event, or they may, in some areas of the body, develop gigantic sizes, a kind of a localized hypertrophy or hyperplasia. For instance, you have all noticed in older people the gigantic hairs that appear in the eyebrows and also in the nose. John L Lewis is an example of one having eyebrow hairs that present a sort of visor effect. Why, we don't quite know

To continue this discussion of balancing, it is necessary to refer to the mineral components of the epidermis, as you see them in microincineration preparations There is in a general way a hypermineralization with age, but there are also areas where we find a hypomineralization or reduction in the amount of mineral. The foci of reduction in the amount of mineral are often identifiable in the gross

all for it but thus far in my laboratory we have not overcome the technical difficulties.

Lerm: Your comparisons appear to be of two varieties of skin, those of the newborn and of the eighty-year-old man. Do you have any data as to when the changes occur? Is it at senility or approaching senility or is it at puberty, or when in the life span? I should think that the newborn is, perhaps, a very nice thing to study but that somewhere along about puberty or just afterwards would be also a good point to investigate

Cowdry: Again, I agree with you completely. We studied too few specimens, and we divided them into the very young group and the very old group, not including the intermediate group, as we should have done But our difficulty was to secure specimens. We can't obtain specimens from the face even at autopsy because of disfiguration, and so we had to limit ourselves to specimens excised from persons who had been frozen solid for thirty days, which is the period necessary in the state of Missouri before you can use the material for scientific purposes.

After treatment with the carcinogen, methylcholanthrene, dissolved in benzene, many chromosome abnormalities are met with as Biesele and I have found (3)

The calcium content diminishes in epidermal cancer (5). It is least in human epidermal cancer (0.008 mg /100 mg tissue), and about the same in mouse epidermal cancer (0 009). For embryonic mouse epidermis and normal human epidermis about the same values were obtained (0 016) In normal adult mouse epidermis there was more calcium (0 044) and in old mouse epidermis, still more (0 054). Thus the calcium content of the epidermis increases with age

Carruthers and Suntzeff (5) have found by [illegible]

[illegible] as carcinogenesis progresses, at twenty days and at thirty days, and in the resulting squamous cell carcinoma, the uptake of radioactive calcium is absent There [illegible]actically no [illegible]

[illegible] these were adult mice

Similarly the uptake of radioactive calcium is very marked in bone. Uptake is also greater by the dermis and by the liver than by blood

Interesting age differences with respect to the uptake of calcium by the liver are revealed The young animals exhibit a marked uptake of bound calcium and but little uptake of free calcium, whereas the

But what actually is the case, as far as we can determine, is that there is noticeably more mineral matter in the basal layers. There is less in the spinous layers and very much more, again, in the granular and corneal layers.

Nelson: That is also illustrated by the low calcium content in the keratotic areas, to some extent, isn't it?

Cowdry: Yes, but, you see, we agree entirely, Dr. Levin, except that the microincineration preparations indicate a fairly large amount of mineral in the basal layer.

Marked changes extend through the whole width of the epidermis, as a preliminary to cancer development in it. There is a loosening up of the nuclear structure, so that materials in the nucleus can be displaced, with the ultracentrifuge, though, beforehand, in the normal state, they could not be displaced. This suggests a reduction in intranuclear viscosity, or an alteration in specific gravity of certain nuclear components.

Goldzieher: Dr. Cowdry, did I understand you correctly that in the precancerous lesions the calcium content is low?

Cowdry: Yes.

Goldzieher: Compared with other parts of the senile skin?

Cowdry: But I am including there only the senile keratoses as precancerous lesions. I am not including the nevi.

Goldzieher: Yes. I was talking about the hyperkeratotic lesions Is the amount of potassium the same or is it increased? One might suspect that the calcium goes down and the potassium goes up.

Cowdry: That would be the logical assumption, but in our microincineration preparations, we can't estimate the potassium at all accurately; in fact, they are of practically no value for potassium. It has been said that the potassium gives a finer ash, an ash that is slightly bluish when viewed in the dark field compared with the calcium or magnesium ash, but to tell them apart is not feasible I admit that the technique of microincineration is tricky, but it is the best we have for the intracellular localization of mineral residues after incineration at about 600° C.

Simms: Dr. Cowdry, I might mention, along that line, in connection with the calcium, that we found in tissue cultures of adult skin epithelial cells, the best growth occurs when the calcium is low and the phosphate is high in the culture medium (27) It would appear that the cells have an unusual affinity for calcium, and the maximum growth occurs with a calcium content about a third that normally present in the blood plasma or serum.

Cowdry: Certainly the culture method can be very useful, and I am

speed of development of epidermal cancers are approximately the same. That is, an age factor does not appear.

On the contrary, a similar experiment on old and young New Buffalo mice yields entirely different results. Both the incidence and the speed of development of the tumors are definitely greater in the young than in the old. The age factor is not suppressed by the genetic factor or factors.

These observations are summarized in Table IV. Examination of this table shows that the influence of genetic strain is also greater than that exercised by the difference in the amount of mineral in the epidermis (10).

One major difficulty with studies on epidermis is that the cells are spread in layers It is necessary in the epidermis to determine as a control the relative proportions by weight of the keratinized layer, of the granular layer, of the spinous layer, and of the basal layer before one *can interpret* changes in the results of chemical analyses since altered chemical composition of the whole could arise from an alteration in the proportions of the various layers and not be indicative of any alteration in the conditions of cell life in any particular layer. This check has been made by one of our people from Siam, Dr. Banyen.

TABLE IV

Influence of Age and Genetic Strain on Epidermal Carcinogenesis

	Young NB 2-3 Mo	Old NB 12-13 Mo	Young CBA 2-3 Mo	Old CBA 12-13 Mo
% Tumor Production	92.8	61.5	69	64.5
Average Time in Months	3.3	4.6	6.3	5.7
% Squamous Cancer	95.6	85	97	97
Normal mgm Ca per 100 mgm epidermis	0.043	0.054	0.040	0.055
Reduced to	0.022	0.021	0.031	0.033
% Reduction	58	61	23	40
Mgm lipid per 100 mgm dry wt epidermis except*	5.25*	32.2	29.3	32.2
Reduced to	3.23*	10.1	15.0	14.7
% Reduction	58	69	48	60

*Total lipid protein nitrogen ratio.

old show a considerable uptake of bound calcium but no uptake of free calcium. This, again, is the work of Lansing and his associates

Table III summarizes briefly the properties of squamous cell cancer compared with those of normal epidermis of mice. In some properties, such as ascorbic acid content, there is no noticeable difference. In others, like water content, there is an increase of less than 50 per cent. In still others the increase is greater than 50 per cent. And there are also decreases listed in the chart of less and more than 50 per cent. Stated differently, the properties of the squamous cell cancer are strangely askew compared with those of normal epidermis (9).

The properties of epidermal cells in neoplasia, in aging and with respect to hereditary control of their responses are very much involved.

In old CBA mice and young CBA mice treated in exactly the same way, i.e., with methylcholanthrene and benzene, the incidence and the

TABLE III

Properties of Squamous Cell Cancer Compared With Those of Normal Epidermis

INCREASE: (50% or more)	Lipid phosphorus: dry wt ratio, succinic dehydrogenase activity, adenylpyrophosphatase activity, arginase activity, lysine, tryptophane, isoleucine.
INCREASE (less than 50%)	Water content, choline, leucine, methionine, valine, phenylalanine, threonine
NO CHANGE	Ascorbic acid NP ratio, specific activity of P^{32} in phospholipid fraction, inositol (wet wt basis), histidine, glutamic acid, cystine, arginine
DECREASE (less than 50%)	Potassium: NP ratio, sodium NP ratio, magnesium NP ratio, cytochrome oxidase, desoxyribonucleic acid (wet wt. basis), cytochrome c, paraminobenzoic acid (wet wt basis), vitamin B_6 complex (wet wt basis)
DECREASE (50% or more)	Calcium NP ratio, iron NP ratio, copper NP ratio, zinc NP ratio, nonprotein nitrogen (wet wt basis), urea (wet wt. basis), ammonia (wet wt basis), total free amino acids (wet wt basis, chromatography), biotin (wet wt. basis), Ca^{45} uptake and retention, free calcium
	NP = Nucleoprotein Phosphorus

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from sixty to ninety, we found a pretty good correlation between the age and the width of the epidermis; in other words, with increasing age, progressively fewer layers of the epidermal cells were seen with the lowest values in the eighty-year-old group (17, 18). In the course of clinico-endocrinological studies of these patients, we noted an apparently close correlation between ketosteroid excretion and epidermal width; subsequently, we found, however, that the correlation actually is closer between the age of the patient and the ketosteroid excretion, hence the correlation between epidermal width and ketosteroids is an indirect one.

Our experience seems to be somewhat at variance with data presented here according to which there is not much difference in the width of the epidermis between the older and the younger group.

I am ready to admit that the skin of every old person does not show thinning out of the epidermis commensurate to the age, but in general, in the older group, the epidermis thins down to three or four cell layers Moreover, the thinning out of the epidermis is usually associated with disappearance of the epithelial cones which project into the cutis and help to form the papillae. Hence the baseline of the epidermis appears to be perfectly straight I wonder whether that corresponds to your experience

Cowdry: To some extent, it does. As you mentioned, we did not find a marked and very uniform decrease in thickness with aging Our measurements were limited to epidermis from the antecubital space. We did find, however, a considerable difference between individuals. There was one old gentleman, I think he was eighty, who had a young skin, believe it or not It was definitely a young skin. That is one of the remarkable things about studies on aging—the persistence of youthful properties All of our biopsies were made from apparently healthy, yet mentally defective, people

Hamilton Dr Cowdry, with regard to growth, we were studying the effect of aging upon the rate of growth and the regenerative interval which can be tested in hairs rather easily, and I wondered how these data compared with those for the epidermis as a whole First we couldn't see any effect of aging upon the regenerative interval, *that is*, the interval between the plucking of a hair and the reappearance at the skin surface of the regrown hair

s a diminution with age. Have you

wish we had The studies on epidermis are complicated, because in aging there is a decrease in number

Nelson: What is the fate of the nuclear material in the epidermal cell, that is, after it ages and passes outward from the basal layer? Is it lost completely or is it simply disorganized? The cornified layers have no staining properties?

Cowdry: What happens is that in the basal layer, the nuclei are robust, rather small, and contain a lot of nuclear chromatin; in the spinous layer, they are larger, and are stained less strongly; in the granular layer, they are smaller, flattened, seem to have more chromatin in them because they are shrunken; while in the corneal layer, the nuclei disappear.

Stern: Did you do any particular studies on melanin?

Cowdry: No, we haven't made any particular studies on melanin. The only thing we have been doing with melanin has been to make an experiment or two which seems to indicate that you can collect the melanin very easily by washing it out and centrifuging it.

Levin: I should think that the calcium uptake, which is so marked in the epidermis as compared to the liver and other tissues is a most interesting phenomenon and I am sure you are probably following it up

Cowdry: Yes, that is being followed up

Levin: And also the differences in bound and free calcium. I certainly would like to know why the skin is taking up all this calcium.

Cowdry: I would like to be able to supplement these studies on calcium by studies on magnesium, but we haven't got that far yet. This uptake work that I am trying to explain has been done by Lansing and his associates. Carruthers and Suntzeff (5) deserve credit for the polarographic determinations and Roberts and Frankel (28) for the amino acid studies It is difficult for me in this informal presentation always to give the proper credit where credit is due to the people who are working in our laboratory

Oliver: May I ask a technical question? We are at present separating and counting nuclei in the kidney and liver. I wonder if, just in a word, you could say how your prepare your cell suspensions

Cowdry: It depends, Dr. Oliver, on whether or not you want to make a chemical analysis of the nuclei. If you want to make a chemical analysis of the nuclei, you can't use the citric acid that we employ for some of our preparations. The only thing you can do is to emulsify the tissue in a homogenizer and try to separate out the nuclei by centrifugation in a fluid which gives you as little loss of nuclear substance as possible.

Goldzieher: I should like to ask two questions: One pertains to the width of the epidermis. In our studies which were carried out on biopsies removed from the inner surface of the thigh of people aged

Horvath: I don't recall the type of analyses that were done, but I know they were done. I think Benedict did some on that a long time ago; I think primarily lipo-protein

Levin: Yes, that is what I would expect.

Nelson: There is a curious point, it seems to me, in this calcium thing that I haven't quite puzzled through, and perhaps you can help me out. With age, you get an increased calcium concentration in epidermis—

Cowdry: Total epidermis.

Nelson: That's right, and, in general, where you get a hyperkeratotic type of lesion, with lots of cornification, you get a low calcium.

Cowdry: That's right

Nelson: Now, if you take that at its face value, this suggests that viability and rapid growth are associated with high calcium. But that doesn't carry through to the age situation What does that result from? Do you have in aged epidermis, for example, a higher proportion of nucleated cells, with a greater viability but a slower rate of turnover? Do I make myself clear?

Cowdry Yes, you make yourself clear, but I just can't answer you That's the trouble

Nelson: Again, in cancer growth, you find a high rate of regeneration and a low calcium

Cowdry: Yes Of course, in the senile keratotic lesions to which you just referred, if they are like those that we have studied, there has been some demineralization We should, obviously, make our examination more comprehensive and include the different types of senile keratosis as well as the nevi

Nelson Is there any way of estimating the rate of epidermal cell replacement? I mean, what is the "half-life" of epidermis cells from young, as compared to old animals?

Cowdry: I thought that was the sort of thing Dr. Jacobson was going to tell us about, in relation to cell division

Nelson. Does the epidermal cell in the youthful skin have a short half-life and is it replaced quickly, and quickly reach the periphery as compared to aged? To some extent, it seems to me that you might reconcile the calcium figures with that, but it is a tenuous bit of reasoning.

Cowdry: It could be. I wish I knew about that. Our ignorance is impressive We don't know how much the increase or decrease is a function of death rate or of birth rate of cells I am eager to find out

Nelson We are planning, and I don't know if it is going to be successful or not, but we are hopeful that we can get a sulfur label in

of hair follicles. The hair follicles are growing outward at a definite cycle but not in unison, as you know. With the dropping out of hair the substance of the hair follicles shifts to the surface and becomes hair of the epidermis. We wish that we could obtain data on the composition of hair follicles. One difficulty is that thus far we have not been able to collect them *en masse* in a condition suitable for analysis.

Hamilton: It brings up another question, if I may continue, with regard to these waves of hair growth which, as you well know, come periodically in the mouse but at longer intervals with aging as Butcher (4) and Parnell (26) have worked out rather well. As I was sitting here, I was wondering, could there be any similar growth waves in your data? The reason I ask that is because of the Scandinavian work, where cancer tendencies are greatly pronounced at the time of these growth waves. Could the growth waves influence many of the things you are doing?

Specifically, I think they affect far more than skin The second hair growth wave in a rat, for example, accompanies the maturation of the reproductive system, that is, the appearance of ovarian follicles, and to some extent the appearance of sperm. Apparently some bone changes also occur simultaneously. I was wondering how these cyclic waves of growth in epidermis enter into this picture

Cowdry: You are speaking about epidermis and not dermis?

Hamilton: Yes, but I think it is a general body-wide phenomenon.

Cowdry: It could be. We must bear in mind the twenty-four-hour rhythm in the multiplication of epidermal cells, the number of dividing cells being approximately double by night what it is by day, so we take the tissues for chemical analysis at the same time always.

Hamilton: No, the waves I had in mind were many days apart. They occur at about the 17th, 36th, 55th and 80th day in the rat, for example (4).

Cowdry: We haven't taken them into consideration We should.

Hamilton: They are tremendously important They throw all of our calculations out of gear when they happen.

Cowdry: It is not going to be easy, but we want to check everything we can.

Levin: Before you go on, may I ask one more question? Have you or anybody else made mineral analyses of hair and nails?

Cowdry: We haven't.

Horvath: Several people have made analyses of nails. Benedict did.

Levin: Is there any notable amount of calcium there such as Dr Cowdry finds in epidermis?

glands, especially in the young, but nevertheless, the chemical hazards are so great it is not surprising that we have modifications in the cell life, perhaps mutations, some of which are possibly malignant.

What is remarkable is that the layer of basal cells continues to produce epidermis for such a long, long time. These basal cells are a very widespread fountain of youth in the body.

And now we can pass to the dermis, if you think we should, Mr. Chairman, or we might—

Oliver: Just one more question, please Have you made any study of the transformation of the granules to keratin, the maturation, one might say, of the granular material?

Cowdry: No That is the purpose of collecting the keratin and attempting to analyze it *en masse*, in relationship to the condition and the age of the epidermis.

Goldzieher: May I say something about the keratohyalin granules? In our studies (17) we found a pretty good correlation between the diminution of keratohyalin granules and the width of the epidermis of the senile skin When various steroid hormones were applied topically to the skin, some steroids produced regeneration of the epidermis as expressed by increased width and reformation of epithelial cones between the papillae This was accompanied by a great increase in the amount of keratohyalin granules approaching the normal level. The amount of keratohyalin present was estimated not only histologically in sections but also by spectrophotometry of reflectance spectra The data obtained by the two techniques were in good agreement and seemed to show that granule formation is an integral part of the regenerative process and represents a function of the epidermis which is reduced or arrested in senile atrophy.

Oliver. Is there not some work that was done in the past that describes the formation and transformation of these granules into keratin? I am very ignorant in the matter.

Cowdry I think in that classical volume of [illegible]

[illegible]

Cowdry: No recent work Presumably, there are two different kinds of keratin here and it is necessary to [illegible]

[illegible] that is laid down? Is it in the [illegible] cell where viability is high, and then succeeding changes are primarily those of a physical nature, let's say, and in secondary terms

some of these basal cells and get a measure of the rate of dilution of that sulfur as it moves outward. Whether or not that will help us, I don't know.

Cowdry: That is exactly the kind of information that should be brought before this group, if you can get it.

Nelson: That is only in prospect, however.

Cowdry: Well, most of this work is in prospect.

Lerm: Couldn't you get an idea as to the rate at which the cells move out by administering a test dose of radioactive calcium and then making radioautographs of selected portions of the epidermis at timed intervals?

Nelson: I think one can. I think it is worth a trial.

Cowdry: It is a good suggestion.

Horvath: Do you find more calcium in the nuclei than in the cytoplasm of cells? You have separated out some of the nuclei of the cells and analyzed them, too, haven't you?

Cowdry: No. We have not made calcium determinations of naked nuclei *en masse*. We have perhaps been unduly fearful that in the collection of the nuclei, calcium would be lost.

Nelson: Well, does your microincineration give you enough resolution to answer that?

Cowdry: The microincineration reveals some mineral within the nuclei.

Nelson: You can't localize it with respect to the cellular elements.

Cowdry: Not well enough to say definitely. I think that in some conditions, there is more calcium in the nuclei than there is in others. But I am not able to give an accurate answer to your question.

Oliver: By and large, the nuclei have less calcium.

Cowdry: Yes, by and large, they do. Now, before we go on to the dermis, I would like to recall very briefly the conditions of cell life in this epidermis We have to look upon the epidermis over most of the body as a sheet of tissue, about as thick as a sheet of carbon paper. Carbon paper measured in St. Louis is about 40 microns thick

The outer half of the epidermis is dead; the inner half is alive. This we must not forget. The inner living cells are exposed to hazards far greater than those to which any other cells of the body are exposed These hazards do not need to be mentioned because they are so obvious There are mechanical hazards; there are physical hazards such as radiation, sunlight, and so on, and there are temperature hazards The epidermis is subject to a greater range of heat and cold than any other tissue of the body. There are likewise chemical hazards. The epidermis is somewhat protected by the oily material derived from sebaceous

immunochemistry of the skin, we thought we would have a lot of concern with particular epidermal participation in it, but the further we go, the more we feel that the epidermis is merely an incident in the whole affair and is only one of the shock tissues, to use a term the dermatologists use a good bit, and may not play nearly as vital a role in the whole picture as we once thought. As you know, in the past a great many people have believed and still believe that the epidermis itself may be the point of origin of antibody. Also, it has been thought that it may be the point of linkage of the hapten with suitable protein to become antigenic. It can be shown experimentally that the epidermal proteins are not required to form an effective antigen, so from that standpoint, the epidermis falls out of the picture to some extent.

It can be shown, I believe, also experimentally, that though antibody is produced in the epidermis, it is at least not the only site of production of antibody You can produce a comparable response so far as the biological evidence of reaction is concerned by a variety of different points of introduction of hapten. This, together with some of the transplant experiments, for example, that have been done, support this view I am thinking of Haxthausen's work with identical twins, in which he took a sensitized individual, one of a pair of identical twins, and transplanted comparable areas, that is, did a cross-transplant. He discovered that sensitized epidermis or skin in the unsensitized host became nonsensitive, and, as the reverse of that, the unsensitized skin from the other individual, transplanted to the sensitized twin did become sensitive Therefore, more and more we are coming to believe that perhaps we are dealing primarily with a visceral source of antibody production.

I don't think we can exclude the epidermis as at least one point of production, but it may not be an essential one Also, we have found ways of modifying the type of reaction so far as tissue changes are concerned, depending on whether you put the material on percutaneously or intradermally, so we are coming more and more away from our original thinking that the epidermis is anything but a reflector, if you will, of these factors. We have found very little evidence for the old view that the epidermis is a source of antibody production and that there is a rapid dissemination throughout the epidermal tissues by some not very clearly defined process of intercellular transmission. Therefore we have come to the conclusion that we must maintain side by [illegible] with the possibility of [illegible]
more [illegible]
and t[illegible]
[illegible] [r]esponsive tissue

It is [f]ound, of course, in aged people, that many of them give a

in a chemical sense? Certainly, the granules become apparent fairly late in the life cycle, but that does not mean necessarily that, metabolically speaking, that is where the chief production of keratin substrate occurs.

Cowdry: I don't think it does. In my opinion the substrate could exist for quite a long time.

Nelson: Much earlier, yes.

Cowdry: In this basal layer, of course, these cells are intermitotic. Their life extends through to the next cell, and they don't become senile and die. The protoplasmic stream is continuous. The cells out in the spinous layer, too, become senile and die; so there is a difference in the manner of life of the cells.

Nelson: The elaboration of such an extensive protein as keratin and keratohyalin material must have occurred fairly early, it seems to me, in the life cycle.

Cowdry. Yes, and also we have to reflect on the fact that our microscopic preparations tell us extraordinarily little about the chemical composition of the tissue we are studying.

Hamilton: Dr Cowdry, Professor Giroud was in America for this conference on hair, held by the New York Academy of Sciences, and his article will appear in that monograph (16). He dealt with histological differences between what he calls the hard and soft keratins, and that is correlated, apparently, with these alpha-beta keratin differences studied by Astwood It is described at length, with pictures, in his article

Cowdry: That will be very helpful Have you any more remarks you would like to make about this, Dr Nelson?

Nelson: No

Jacobson: Dr. Cowdry, may I ask you a question about the chemical nature of the keratohyalin granules? Do you think they contain a ribonucleoprotein, as has been suggested previously?

Cowdry: I don't know yet They might It is for Updyke who can collect them *en masse* to find out I think they have a good deal of sulfur. I would like to ask Dr Nelson whether studies such as he is carrying on in immunochemistry could, perhaps, give us some indications for our work on the aging of the epidermis

Nelson: It should be stated that the work going on in our laboratory* is not directly related to aging, but is concerned with the immunologic mechanisms of cellular sensitivities and, in particular, with skin sensitivities. This work is being done by Dr. Herman Eisen, not by me. When we first became concerned with the problem of the

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immunochemistry of the skin, we thought we would have a lot of concern with particular epidermal participation in it, but the further we go, the more we feel that the epidermis is merely an incident in the whole affair and is only one of the shock tissues, to use a term the dermatologists use a good bit, and may not play nearly as vital a role in the whole picture as we once thought As you know, in the past a great many people have believed and still believe that the epidermis itself may be the point of origin of antibody Also, it has been thought that it may be the point of linkage of the hapten with suitable protein to become antigenic. It can be shown experimentally that the epidermal proteins are not required to form an effective antigen, so from that standpoint, the epidermis falls out of the picture to some extent.

It can be shown, I believe, also experimentally, that though antibody is produced in the epidermis, it is at least not the only site of production of antibody You can produce a comparable response so far as the biological evidence of reaction is concerned by a variety of different points of introduction of hapten This, together with some of the transplant experiments, for example, that have been done, support this view. I am thinking of Haxthausen's work with identical twins, in which he took a sensitized individual, one of a pair of identical twins, and transplanted comparable areas, that is, did a cross-transplant He discovered that sensitized epidermis or skin in the unsensitized host became nonsensitive, and, as the reverse of that, the unsensitized skin from the other individual, transplanted to the sensitized twin did become sensitive Therefore, more and more we are coming to believe that perhaps we are dealing primarily with a visceral source of antibody production.

I don't think we can exclude the epidermis as at least one point of production, but it may not be an essential one. Also, we have found ways of modifying the type of reaction so far as tissue changes are concerned, depending on whether you put the material on percutaneously or intradermally, so we are coming more and more away from our original thinking that the epidermis is anything but a reflector, if you will, of these factors We have found very little evidence for the old view that the epidermis is a source of antibody production and that there is a rapid dissemination throughout the epidermal tissues by some not very clearly defined process of intercellular transmission Therefore we have come to the conclusion that we must maintain side by side with the possibility of epidermal production of antibody, the probably more important possibility that we are concerned with a visceral source, and that the epidermis is merely a responsive tissue.

It is found, of course, in aged people, that many of them give a

different response in terms of the efficiency with which one can produce sensitization with simple haptens, and, as compared to young people, there may be a considerable difference in terms of the clinically or histologically observed reaction thereto. I don't think, however, I am competent to discuss that.

Cowdry: The epidermis is a kind of happy hunting ground for viruses. Are antiviruses or antibodies for the viruses to be included or not included in our concept of epidermal function?

Nelson: I don't think we have anything to contribute to that, Dr. Cowdry. We had felt originally, probably because it is one of the currently accepted thoughts, that the epidermis must be a greater source of antibody generation than, perhaps, it is.

Cowdry: Of some type.

Nelson: Yes, of some. We see no reason to believe at the moment that it is the only source or even the most important source.

Cowdry: Is there any discussion of what Dr Nelson has just said? If there is not, we shall go ahead with the dermis. If you think of anything later, please bring it up.

The dermis is a mixture of cells and fibers and tissue fluid in which both are located. There are present in the dermis also blood vessels, lymphatics, and some sense organs. The dermis shows marked regional differences in structure, as does the epidermis In general, we could contrast the dermis with the epidermis by saying, first, that it is not so richly cellular. The epidermis is, perhaps, the most cellular tissue in the body. The dermis is relatively easily separated. The cells of the epidermis, being extremely closely bound together, are very difficult to separate. The epidermis is avascular and alymphatic, whereas the dermis has both of these kinds of vessels Above all, the epidermis does not possess the large amount of intercellular material that the dermis has. In this intercellular material we recognize interesting components.

Before we take up the two categories of components, the fibers and the substance between them, the tissue fluid, it might be well to say that there is a considerable variety of cells in the dermis They are extremely difficult to unravel. They are mostly of the mesenchymatous type, and the mesenchymatous cell is like an actor who changes his appearance depending upon what he wants to do. These mesenchymatous cells change their appearance depending upon whether or not they are applied to a surface, and in relation to a great many other events in their lives. They change their appearance, so that there is really no agreement even as to the classification of the mesenchymatous cells. We can't collect them in large numbers from dermis for chemical

analysis because they are relatively few, and because to separate the different types is not easy. There is only one type for which we have pretty definite information, and that is the mast cell. The mast cell, as you know, is characterized by having large basophilic granules. Kulonen has recently published a paper on the decrease in number of mast cells with age in the epidermis (23). It is assumed that these mast cells have something to do with the production of the material which occupies the space between cells and fibers It is also assumed that the fibroblasts have a good deal to do with the production of this material. We need more information.

The material between the fibers in the tissue fluid is subject to great local or regional differences It also differs with age. In one of these papers, some of the differences with age have been mentioned; that is, the increases with age. We would like to get further information about it. According to McMaster, you cannot get free fluid from dermis without profound injury to the dermis. The material is present in the form of a gel I think the best introduction to this material is contained in the series of papers published by the New York Academy of Sciences (13).

To my mind, the discovery of this gelatinous material between the cells is very interesting. I have particularly in mind the work of Dr. Sylvia Horton Bensley (2) She created an increase in the amount of tissue fluid, and trained paramecia, so that they could stand the change in environment that took place on entry into this tissue fluid from water. As they moved around in this tissue fluid, she noticed that they occasionally came up against obstructions in the fluid which she could not see microscopically. From this observation she deduced the existence of areas of tissue fluid of gel-like consistency.

The presence of a gel impedes likewise the spread of materials in the tissue fluid of the dermis and consequently to some degree conditions its permeability.

At about the same time came the very significant work on spreading factors, done principally by Duran-Reynals (13) and his associates This showed that the gel-like background of connective tissues can be broken down by an enzyme. The principal enzyme is hyaluronidase, acting on hyaluronic acid. Marked differences have been reported in the ability of material to *spread*. With advancing age of the dermis the spreading is lessened There are also regional differences in the ability of material to spread in the dermis. To summarize, we are trying to incriminate certain types of cells in the production of this intercellular material which is a complex polysaccharide, or group of polysaccharides, and to study the enzyme or enzymes which break down

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the barrier and in so doing increase the permeability of the dermis. Much has been written on these subjects. They constitute one of the most active fields of investigation we have at the present time.

To continue, I would like to refer to the fibrous components of dermis. The fibers in the dermis are of two main types. The first is collagenic, and the second is elastic. There is a supplementary type called *reticular*, which is, we think, really a modification of the collagenic.

Now, *mentioning the work done by Lansing and Rosenthal and* Roberts and Carruthers and others, it is possible, as you know, to analyze or to extract the elastin from the arterial walls (24). The same methods have been used to extract elastin from dermis In the arterial walls, the young and the old elastic fibers appear different (15). They are straight and anastomosing, they are glistening, and they are water-clear in the young In the old, they are frayed, senile, yellow and dull, and tend to clump. The tinctorial methods are fairly obvious, but perhaps not of as great value because they are not so discriminating, that is, specific Age differences in the properties of human arterial elastin are summarized in Table V.

The chemical differences are also shown in Table V. Arterial elastin from the young is substantially mineral-free In the old, it is heavily mineralized. It is phosphorus-free, too, in the young, but in the old there are large amounts of phosphorus That brings up the question that was asked of me in regard to phosphorus in the epidermis The amino acids are rather strikingly different There is a trace of aspartic acid and 1 per cent of glutamic acid in young elastin Paper chromatographic methods do not show the aspartic acid but the glutamic acid appears In older elastin, the chromatographic records demonstrate glutamic acid conspicuously, and aspartic acid is clearly revealed

Simms: What are the age groups?

Cowdry: The age groups are given in Lansing's paper (24) I am not quite sure, but they are between young adults and persons of seventy and eighty. There was a considerable age difference

Simms: But the young are adult?

Cowdry: Yes, the young are adults That brings up this question of whether the elastic fibers of a young adult are mineral-free I have my doubts about whether that is the case throughout the circulatory system, but I think it might be the case in some areas My impression is that minerals, particularly calcium, come in fairly early in young adulthood.

Oliver: May I ask, how you get the elastic tissue out of the wall of the aorta? Is it an extract, the result of a chemical extraction?

TABLE V

Some Properties of Human Arterial Elastin

Properties	Young	Senile
Physical appearance	Straight, *anastomosing* threads or ribbons Glistening, refractile, water clear.	Frayed, fragmented, thin strands and granules Tend to clump, yellow, dull
Tinctorial	1 Not stained by hematoxylin 2 Red color with Congo Red 3 Resorcin-fuchsin, orcein, "Van Giesen" positive	1 Take up hematoxylin 2. Yellow-orange with Congo Red 3 Stain more densely with resorcin, etc.
Chemical	1 Mineral-free 2 Phosphorus-free 3 Amino Acids· a) trace of aspartic acid b) 1% glutamic acid	1 Severely mineralized. Contains as much as 14% calcium. 2 Large amounts of phosphorus 3 Amino Acids a) appreciable amounts of aspartic acid b) 4% glutamic acid
Physical	Dry isolated elastin, has specific gravity less than 1 30	Dry isolated elastin, has specific gravity greater than 1 30

Cowdry Yes, it is by chemical extraction

Stern Is it known of what nature or what type that phosphorus is? Is it known in what compounds it appears?

Cowdry The data here presented were for total phosphorus

Stern. But you do not know whether it is from lipids mainly or from what type of compounds?

Cowdry: I think that is made clear in Lansing's papers There is a certain amount of lipid, especially in the older arteries There is probably a mixture of all kinds of lipids, including cholesterol

The amino acid composition of arterial elastin changes with age (24) There is a significant increase in the aspartic acid, which I have mentioned already In the young, it is about 14 per cent, and in the old, 1 5 per cent That is approximately a tenfold increase The increase in the glutamic acid, too, is rather conspicuous It is interesting that the other amino acids, represented in the chromatograms, do not

the barrier and in so doing increase the permeability of the dermis Much has been written on these subjects. They constitute one of the most active fields of investigation we have at the present time.

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narily, and to the cells therein, the fibroblasts, the mast cells and others That brings us to the fibroblast.

If anyone can distinguish an old fibroblast from a young fibroblast, it is only a superficial kind of distinction and an individual matter between different fibroblasts and the tissue culture. If we take from an aged person fibroblasts and grow them, and take from a young person fibroblasts and grow them, the fibroblasts which grow in both instances are perhaps indistinguishable. We would like to get more data on that sort of thing, and possibly some slight differences in their appearance are due more to environment than to inherent differences. In our problems of aging, we have to admit, I think, that some kinds of cells in the body retain their youthfulness as long as the body lasts. We are referring to kinds of cells, not individual cells. The cells in the bone marrow that produce the formed elements and the hemoglobin-containing red cells, apparently retain these youthful properties. The cells in the epidermis, in the basal layer and in the spinous layer, perhaps, retain their youthful properties That is, again, the kind of cells do, not the individual cells Some epithelial cells of the intestinal tract do likewise In aging, therefore, we think that the tissues composed of cells which do age and which don't age form an interesting contrast

The difficulty is, again, to separate the groups of cells which show differentiation and aging from the groups of cells that do not To collect fibroblasts, for example, in a manner suitable for chemical analysis has been something that has defied us for a long time and still defies us We don't know how to go about it. If you have any suggestions, they would be most welcome

Now, I have completed, as far as I can, the presentation of the aging of the integumentary system You will see that I have not included a great many things I have not included the integumentary appendages, as I should like to call them, namely, the sweat glands and the sebaceous glands I have not included the hair because we have a specialist on hair present here and I don't want to expose my ignorance too acutely for you to see It seems to me that there are few structures in the body that are quite as beautifully adapted for studies on aging and the [illegible]
fluence of endo [illegible]
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means o [illegible] is the object of these conferences. Will you carry on, Dr Hamilton?

Hamilton: Well, I don't know that I am a specialist in hair I guess that is a little bit like the physicist who finds that the physicists say he is a chemist and the chemists say he is a mathematician and the mathe-

show alterations with age of the same order of magnitude at all. The glycine, valine, proline and so forth remain fairly uniform. Therefore, as far as amino acids are concerned, there is an asymmetrical kind of alteration.

Now we come to the problem of what this means, and your interpretation is of great interest to me and also to Dr. Lansing. There are so many factors involved that it is difficult to speak with assurance about anything. Probably the best way is to get some distinguished individual and disagree with him and then let him convince you that you are wrong and he is right. Then you sort of bring out the facts. What Lansing thinks, I believe, is that in the arterial wall and also to some extent in the dermis, where we have the same two types of fibers (the elastic tissue and the collagenic), there is a change in the concentration of these amino acids that is in some way linked up with the fixation of calcium; that the calcium then is bound and incorporated into the substance of the elastic fibers, as can be seen in histochemical preparations. Why this should occur with age is not so clear, of course. And why it should occur in certain segments of the circulation much more markedly than in other parts of the circulation is also not clear. There was a little discussion about that yesterday What is primary and what is secondary here we cannot tell except that it seems to us that the protein changes are likely to be the forerunners of the mineral changes. But again that is a little difficult to be sure of.

So much for the elastic tissue Let's consider the collagenic tissue now Collagenic fibers have been subjected to study by Schmitt and others (20, 29) with the electron microscope, and it has been found that instead of being homogeneous, they are banded; that the interval between bands is definite and can be measured; that by means of the electron microscope one can therefore acquire some information as to the quality of the collagen which was not feasible before, just as one can acquire information about the quality of steel The whole subject of the collagenic fibers, it seems to me, is extremely interesting and ripe for development in any program on aging.

There are the individual differences that we know in physical state, in life expectancy, and in a great many other things These may be partly due to differences in both elastin and collagen. Perhaps we can be said to have good collagen as we have been said to have good or bad rubber. Certainly, it should be possible to make progress in this direction by contrasting the physical properties and chemical properties of the collagenic fibers in different parts of the body where aging is rapid and where the aging is slow, and at the same time to give considerable attention to the tissue fluid background, which we think is a gel, ordi-

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Hamilton· Well, I don't know that I am a specialist in hair I guess that is a little bit like the physicist who finds that the physicists say he is a chemist and the chemists say he is a mathematician and the mathe-

matician doesn't know what he is. As regards hair, precisely, we would be more interested in its genetic and endocrine and aging relations than, perhaps, in the structure itself. As regards aging—

Cowdry: Just a moment, about the genetic. It seems to me that in the epidermis, we have a tissue which is most exposed to the external environment and also is pretty firmly in the grip of genetic factors, whereas, with the internal tissues, we have fairly adequate protection from the external environment coupled with genetic control. To contrast them may bring out the effects of environment.

Hamilton: I might, if you care to have me do so, Dr. Cowdry, throw out an idea for discussion here.

Cowdry: Please do. That is what we want you to do.

Hamilton: We have been interested in baldness Chronologically our work progressed as follows: We found that the eunuchs who were our subjects did not develop acne if they were castrated before puberty, but some of them would if they were treated with male hormone substance. It was a very clear-cut phenomenon, because sebaceous secretions would increase, it was histologically evident, and you could collect samples from the skin that showed an increase. Papules would form and become pustules. We never saw the big, indurated lesions that one sometimes sees in acne, but the small acneform lesions did appear, and they were related to androgens, because, first, one could get a papule to develop, then stop androgenetic treatment of the eunuch and get that papule to become wizened and fade away. If treatment were instituted again, say, in two or three weeks, the papule would again *perk up and go on and become pustular*

Then baldness was found to have a similar relation to androgens. As it worked out, there were three factors The first was a genetic factor. The subject had to have the proper ancestry. No amount of androgens would produce baldness if the subject didn't come from a baldness-susceptible family. The second factor was androgens, and they were not effective without the genetic aspect, and the genetic aspect was not effective without the androgens One could have a person from a family in which all the male members became bald but if these people didn't mature sexually, they did not become bald; so there were two interdependent factors The third factor, equally dependent, was aging. If a young eunuch, say, a man in his twenties who was castrated prior to puberty, is given androgenic treatment in the third decade of life, he will get bald at about the same rate that young men will over the course of several years On the other hand, if one treats a man who is sixty years old, and has been castrated since prior to puberty, he will get bald very quickly, if he is going to develop this condition

Within six months almost all the hair that he will lose is gone There is a very interesting example there of the storing up of greater sensitivity in the absence of the actual stimulating agent.

Well, here are three factors that I think are the same factors, perhaps, plus some extraneous environmental ones that may be operative in many of these genetic-androgen aging conditions; namely, an aging factor, a genetic factor, and a stimulating factor, in this case an androgen I started thinking about this, since it probably wasn't happenstance that two of the most common diseases known, acne and baldness, would have a strong male factor in them, and I started wondering, "Well, just what is maleness? What is this quality, or what is it concerned with?" and that led to the question of longevity and male-selecting pathological conditions

To summarize briefly, there is now a sex difference of about five or six years in life span in this country, the male being that much shorter-lived than the female, and I think it is due, in some part at least, to a maleness factor I wonder if these same three influences that I mentioned for baldness might not be found—and that is what I would like to hear *discussed*—in some of these other male-selecting conditions First, what are the male-selecting conditions? We have a list now, from rather casual reading, of over several hundred of them We picked out the female-selecting conditions, and there are three facts that fit in there The first is that of these many hundred conditions that appear in one sex preferentially (at least at an early age) about two out of every three are those that affect males predominantly, and only about one-third affect chiefly females

Oliver What are some of them?

Hamilton Cancer of the lower lip, floor of the mouth and tongue, coronary thromboses; thromboangiitis obliterans The first fact was the tendency of most of these sex-selecting conditions to be male-selecting conditions The second fact is that the conditions occur almost solely in one sex, namely, over 90 per cent of the cases occurred in males We found only two conditions which occurred primarily in females

Oliver: Which were they?

Hamilton They were congenital dislocation of the hip, and an increase in thickness of the frontal bone

Coudry: Hyperostosis frontalis interna.

Hamilton: Yes Those were the only [illegible] out females

[illegible] y and large,

[illegible] When one

[illegible] per cent or more of the cases occur

in one sex, they are almost all male-selecting conditions.

The third factor that struck me as rather interesting was that it didn't seem to matter which organ system was involved, whether it was circulatory, genitourinary, osseous, or muscular; it seemed to be a general effect, with two exceptions: the conditions that affected primarily the integumentary system were about evenly divided in the two sexes, and those that occurred in the endocrine system (due chiefly to thyroid and pancreatic disturbances) tended to be found more in females than in males. I am talking about the number of conditions The third fact, therefore, seems to be that this is a general phenomenon and not primarily related to the circulatory system or to any other one system. Now, I thought, "That is all very well" —

Simms. Are you talking about mortality or morbidity?

Hamilton. I am talking about incidence, or morbidity. I thought, "That's all very well, just from a little casual reading, and perhaps somebody else doing casual reading would find something quite different," so I turned to the Census Bureau reports — in other words, mortality, now — and I found that almost all the chief killers, in terms of time of appearance, were those that involved primarily the males That work is by Ciocco, published in the *Quarterly Review of Biology* (8). Therefore, we are dealing here, really, not with just casual things, but with the ones that are the major causes of death

The next point is, what is responsible for this? Is it something that comes at the time of maturation in itself in males? Is it of endocrine nature, or is it not? The first thing of interest is that this lesser viability of males extends all through life It is found prenatally, apparently, but the great difference in the incidence of these conditions comes after puberty, so that it does seem to be at least accentuated by sexual maturation.

Engle: Dr. Hamilton, have you looked into that problem of prenatal loss for these sex-selecting factors of yours? The conception rate, for instance, is given as about 140 females to 100 males. What about the prenatal loss? Are there these same selecting factors operating prenatally?

Hamilton: I'm glad you asked that, Dr Engle I think there are 105 males to 100 females, by and large

Engle: At birth?

Hamilton: Yes, at birth; but there is a much higher percentage, as Dr. Engle mentioned, of males among abortions and miscarriages Ciocco (7) has analyzed that, and he divides the census material, for whatever dependency you wish to place upon it, into thirteen causes of death. All of those thirteen were, with one exception—the exception

being congenital malformation—ones that affect males much more than females, so, again, the effect of maleness is a general one. If one looks up the accidental deaths, deaths as a result of trauma and such, one [illegible]

[illegible] :al. The male

[illegible] between birth and one year of age, and I don't think the boy is a swashbuckler at six months of age.

Oliver: The difference, perhaps, isn't as great in that period.

Hamilton: The difference between the sexes in accidental deaths is about twenty-five per cent between birth and one year of age.

Oliver: Well, what is it when they are older and exposed to accidents?

Hamilton: I don't think a boy is more likely to get dropped on his head when he is six months of age than a girl.

Engle: Oh, sure he is. He is going to fall off a table much more often than a girl A girl won't climb up on a table so frequently.

Hamilton: Well, let's just examine that question. It is generally considered that males have a much tougher life, work at the office is much more troublesome than, let's say, the work of the wife who has to meet the bills and pay the butcher and so on. Therefore, all these early tendencies in males come from their greater exposure to troubles. I disagree I think there is a general—

Oliver: Most accidents, I am told, occur in the home.

Hamilton: Yes, but I think there is a general lesser viability of males than of females at all stages of life, beginning *in utero*.

Stern: But there are cultural and psychological factors that contribute to sex differences in mortality.

Hamilton. Absolutely, but I don't think one should explain that by—

Stern: It seems more or less proved that the ratio of peptic ulcer in the two sexes was essentially different sixty years ago from what it is now, so there are definite psychological differences or factors which come into it

Hamilton: I wouldn't doubt a bit that environmental factors add to this [illegible]

Hor [illegible]

a gooc [illegible] who have probably worked as hard, if not harder, than most males ever worked? Do they have higher mortality rates?

Hamilton: We have no such data But I might point to one instance in which inherent susceptibility might be blamed wrongly on environ-

mental factors. Syphilis in the very young—I forget the exact figures, but let's say before three years of age—follows as much a sex-differing course as in the adult male. One explains the worse course of syphilis in the adult male than in the adult female, if you want to use environmental explanations, on the basis of reinfection of males, but the higher mortality rates in males than in females occurs also in the very young boy, in the infant, who is not sexually active.

Horvath: Well, there are more young boys, then.

*Hamilton: So that one would have to multiply all these environ*mental explanations to fit each of hundreds of pathological conditions that affect males preferentially. If you want to adopt that attitude, you have to say, "This causes this, and that causes that." Instead, I think it is much more logical, and it fits the facts better, to see if there is a lesser viability on the part of males May I give one other bit of evidence?

We have tried to look up life-span data for various animal species, and I think on the last count, we had something over a hundred. About 80 per cent of these showed lesser life span in the male than in the female This holds for nematodes, molluscs, crustacea, insects, arachnids, birds, reptiles and mammals (21). Now, maybe the nematode has a harder life at the office, I don't know. Maybe his syphilis, or whatever he has, is worse.

Engle: Dr Hamilton, may I interrupt, because Dr. Heilbrunn over here is getting very restless?

Hamilton: I would like to hear what he has to say.

Engle: You stay right up there because you have to defend yourself. Dr. Heilbrunn, you know all about nematodes.

Heilbrunn: Not very much There aren't as many data as you might expect If you take the lower animals, you have to take special cases. For instance, the male bee has a nuptial flight and then dies immediately after flight, whereas the female has to lay eggs for twelve years. There is nothing general in that phenomenon It is a rather special case. The data that we have are made up of a lot of such special cases. The life span of a lower animal is determined by the necessity for reproduction Once reproduction is achieved in an insect, the insect dies. I think these things should be interpreted rather carefully Actually, as Dr. Hamilton knows, the total data on life span in animals are very meager, and many of the data that have been presented may be viewed with great suspicion.

Hamilton: I agree, and I think a great deal more data should certainly be obtained. As far as the evidence goes, though, the life span of females is longer in these various species throughout the animal

kingdom, not just in vertebrates, but in invertebrates as well. The reason [illegible] animal kingdom in general.

Engle: You really mean lesser protoplasmic resistance.

Hamilton: Exactly.

Engle: I am very much intrigued by the sex differences in mortality, but you must always keep in mind, as has already been mentioned, the cultural factors as well. I think Dr. Stern might have something pertinent to say

Stern: There are two other points on which I should like to comment with regards to Dr Cowdry's presentation. First, to come back to what I began to discuss yesterday, there is this question of local aging, in which we are so interested. in the central nervous system. That brings up the whole problem, which is almost of a philosophical nature, as to what extent these morphological changes are, really, indicative of aging, and what is their functional significance? For example, I mentioned yesterday, to take a very marked contrast, the *Purkinje* cells Let us say, take one morphological index, that is to say, brown pigment, which is generally recognized as a mark of *aging in* the central nervous system If we take two very *contrasting areas*, the Purkinje cells, let us say, and certain cells in the spinal cord, we have no evidence clinically that the cerebellar function is better preserved in very old people than certain functions of neurons with a high amount of brown pigment If we take, for instance, the thoracic cord in the anterior horn cells, there is a tremendous amount of lipochrome in old people, *in* the *lateral* horn cells which are just in the neighborhood and which have something to do with the sympathetic innervation, there is very little brown pigment, and yet there is no clinical evidence that anterior horn cell function is more impaired in senility than lateral horn function of the sympathetic innervation

When you take another *morphological* phenomenon, let's say the accumulation of glial fibers in the cortex, under normal circumstances, except for layer 1, they *don't occur at* all In senility, you see some glial fibrous astrocytes, but the increase of fibrous astrocytes in the anterior horn, in the cord, is much more marked than in the cortex. Clinically speaking, there is actually more evidence of senility of the cortex in senile people than there is of senility of the anterior horn from the point of view of actual function

In other words, how far are we allowed to take any one of those various morphological and chemical factors and conclude from those

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Hamilton: I agree, and I think a great deal more data should certainly be obtained. As far as the evidence goes, though, the life span of females is longer in these various species throughout the animal

would really be most intriguing to do a parallel study.

Oliver: Other than that they are both black, is it known that they are the same chemical substance?

Stern: That this is melanin, you mean?

Oliver: Yes, melanin—malarial pigment is called "melanin" by some. It only means "black."

Stern: No. This, as far as I know, has actually been proved to be melanin, the same melanin.

Oliver: In the same chemical sense, that is, containing sulfur?

Stern Yes

Engle: Dr. Stern, for my information, did you say the *substantia nigra* was present only in humans?

Stern No, no, only the melanin in these cells The *substantia nigra* is a nucleus of the midbrain that appears very early in mammals. I have forgotten just when.

Engle: Yes, but the melanin, you say, does not occur in apes?

Stern: Not in anthropoid apes.

Hamilton: Are you sure of that?

Stern: Absolutely

Hamilton: James Brown of Jefferson Medical College tells me it is in the orangutan and chimpanzee

Stern: [illegible]

I have ne [illegible]

Hamil [illegible] adult or young chimpanzees?

Stern: I don't know.

Hamilton. It comes late in life in the chimpanzee, according to Brown

Stern: I was at one time consulting neuropathologist to the London Zoo and I saw the most extraordinary things there I saw midbrains of apes, but I don't recall what the history was. But Monakow and others who have worked on comparative neurology say that the *substantia nigra* does not contain melanin in apes But perhaps they also took too young animals

Hamilton: Brown's work on that is the only report I know of

Horvath: What about the size of these cells in the areas which are distinctly aging a little faster than others? Are they larger cells in that area or are they smaller cells? For instance, we know that in certain types of pathological disorders, like poliomyelitis, for instance, the large cells are much more susceptible to damage than are the smaller cells Is this also true here? Do we have a separation of large cells in these various tracts versus small cells, and is that where the large cells are more susceptible or is that just—

that there is a diminution of function? I am speaking here from the point of view, at least, of the central nervous system. Sometime ago, there was one of those newspaper clippings in *The New Yorker* magazine. It had the heading, "A Thought For This Week," and it was taken from the advertisement of a shoe firm which said, "A man is as old as his feet." The same thing applies here. You cannot say, for instance, in the central nervous system, a man is as old as his inferior olive, for instance, because the inferior olive is heavily laden with brown pigment It so happens that other areas do not take the brown pigment to such an extent.

Another point I want to bring up is that about melanin. I am particularly interested in melanin because of something which some people do not realize Melanin in the inside of the central nervous system, as we know particularly with respect to the *substantia nigra* and the other melanin-bearing areas like the nuclei of the tenth and certain areas in the hypothalamus which are not studied as well, is specifically human The *substantia nigra* (which is called *nigra* because it is black) is not black even in anthropoid apes. Not only does the melanin appear late phylogenetically but also ontogenetically. A newborn is born with an unpigmented *substantia nigra*. The *substantia nigra* develops a black pigment with increasing age, and it is because the nerve cells in this area contain a tremendous collection of melanin This melanin not only is specifically human, but it appears in man, I think, only around the third or fourth year of life. This has not been very well studied as yet.

I have also been interested, incidentally, in the melanin in senility. I have always had the impression that you find a lot of the melanin pouring out from the nerve cells, free in the tissue, and also in scavenger cells around vessels and so on, in senile brains. But I think that should be studied systematically Nobody actually knows anything about the role of the melanin here. If something is specifically human in the brain and appears so late ontogenetically, and is associated with important endocrine functions, it should have some significance, but we don't know what it is.

I think it would be very interesting to study melanin in the skin, since skin and central nervous system derive from the same matrix embryologically, and in many ways it has even maintained certain features during life which would serve for parallel study — of the melanin in the human nervous system during the life span and also in the skin, because in the skin, too, we have these changes like local melanin collections and so on, and change of melanin distribution with age. And since there is a connection with pituitary function, it

the aged skin the collagenous fibers lose their separate entity and coalesce into a hyalin mass These changes, however, are not specific for the aging tissue of the dermis for they are familiar to the pathologist who observes them in a variety of tissues and conditions. These hyalin changes of the collagenous fibers were referred to as "edema sclerosis" by Krompecher who maintained that they are preceded by an accumulation of interstitial fluid of long standing. The fibrils bathed in the fluid undergo physico-chemical changes—their substance is eventually transformed into hyalin

It is not improbable that an alteration in the distribution of interstitial fluid in the skin occurs also in the course of the aging process and accounts for the characteristic changes of the collagenous fibers of the aged skin.

Another constituent of the skin, the elastic fibrils, also show characteristic changes in the aged These fibers can be studied in the skin almost as well as in the aorta We have studied them with the help of two techniques histology and spectrophotometry. Histological studies clearly show that the fibrils in the aged tend to break up and become fragmented This is accompanied by changes in structure and outline: instead of long, wavy, fine fibrils we find short, straight and ill-defined elements, some of which end in clumps Finally, the tinctorial quality of the fibrils also changes inasmuch as the staining spreads to adjacent collagenous material, a phenomenon referred to by the dermatologist as basophilic degeneration It is quite interesting that these changes are reversible to a considerable extent, for instance, upon topical application of estrogenic hormone. This was shown by histological methods in our studies (17, 18) and by Esben Kirk and his co-workers (6, 22) by actual measurements of skin elasticity Direct spectrophotometry of the living human skin also corroborates the histological findings for it shows a decreased absorption in that part of the spectrum in which the absorption bands of elastin are found.

Heilbrunn: I believe that in the future there will be more work on the physiological changes in age The anatomical work comes first, and then the histochemical and the chemical, but it is possible to gather data on changes in function with age That can be done even with skin. One of my students, for example, took pieces of the skin of newborn rats and was able to measure the rate of restoration very successfully We made no study of aging, but it is possible in some of these systems to make physiological studies as well as more static studies in histochemistry and morphology

Cowdry: I am all for these physiological studies. I think that the goal we have in mind is to determine how function is modified by

Stern: First of all, there is no rule for that. For example, the cells of the column of Clarke in the spinal cord, which are laden very early with this lipochrome or brown pigment, are very large cells; whereas, for instance, in the subthalamic nucleus of Luys, the cells are very small, and they have a strong tendecy to accumulate lipochrome. On the other hand, in Purkinje cells, which are much larger, there is no lipochrome—well, there is no correlation with size of cells but, on the other hand, I would not call this damage. It is not damage in the sense that, for example, the cell changes in poliomyelitis are indicative of damage, because it is actually a physiological process.

Horvath: But suppose even in these very specialized areas where you have different sized cells, that is, one being very small in one group and very large in another, there is still a certain statistical variation in size of those cells. Do the larger cells have a greater amount of this pigmentation or are the smaller cells involved in it also?

Stern: I don't think anyone has really studied this quantitatively, but naturally, in a larger cell you see it much better.

Horvath: Oh, yes, of course

Goldzieher: Can we get back to the skin for a moment?

Engle: Surely.

Goldzieher: Dr Cowdry has raised a question of fundamental significance; namely, whether a cell is aged as such or only by virtue of its environment? This question should be considered in relationship to the problem of regeneration. Some cells of the animal body are capable of regeneration in the adult organism whereas others are not. The nerve cells of the central nervous system and of the eye, for example, do not regenerate but the fibroblasts, the epidermal cells and many other cells do and they do it quite rapidly Such regeneration is observed in the aged as well as in the young though, of course, at a slower rate in the aged.

It would seem to me that if this power of regeneration is inherent in most of the cells of the body and certainly in all cells of the integument and is maintained even in old age, we have to postulate some extracellular factors which are responsible for the changes of aging It seems logical to suspect that the fault lies in the internal environment in which the cells live or in the humoral forces which alter this environment adversely These environmental influences act or fail to act upon the metabolism, or more specifically on the anabolism of the cells, hence with decreased anabolism the power of the cells to regenerate decreases, and morphological or functional signs of aging begin to appear.

Another point I wanted to raise concerns the collagenous fibers In

Hamilton: Well, I don't know of any spectrophotometric evidence that showed that melanin in the neurological tissues is or is not like that in the skin. But in the skin, Edwards showed a melanoid in the outer layers of the epidermis which had this different spectrophotometric appearance from what he called melanin which was located deeper in the epidermis

Stern: I always had the impression that the one in the central nervous system is argentophilic When you do, for instance, a Bielschowsky stain—of course, you don't do it for that purpose, you do it to see the neurofibrils—then the melanin in the midbrain stands out very beautifully. But that, again, has never been studied specifically.

Cowdry: The melanin in the epidermis is argentophilic, but I don't think that means very much; at least, I would not put too much emphasis on it

Shock: I should like to ask one question about skin. As a physiologist, I think of skin as being one of the major places for the storage of water I wonder if there is any information about whether water goes in and out of skin in the old animal at a lower rate than in the young, or has the old animal lost part of its potential storage capacity for water?

Cowdry: You remember the work that Lowry and Hasting did (25). It surprised most of us, because they found that in some tissues there was not a decrease in water but an increase, with age Now, I don't think that these determinations for skin amount to very much, because the skin of different regions is so very different Their determinations aren't determinations of epidermis alone, or of dermis alone, but of both together But the view is generally held, following our friend, Walter Cannon, that the dermis is the place of storage of water. However, the measurements remain to be made in respect to the changes with age.

Shock: Is there any evidence that there are age differences in the hyaluronidase content of tissue?

Cowdry: I think there is. The evidence is fairly good, because you can bring out the difference tinctorially between the ground substance of young and old skin In addition one can measure differences in the actual spread of substances put into young and old dermis Some correlation has been reached between this and the number of mast cells in the study I referred to earlier (23), so the evidence, it seems to me, is substantial But there are always difficulties in interpretation, because there *isn't* just one complex polysaccharide but there are probably two groups of complex polysaccharides, one of them containing sulfur, and various mixtures of the two. The degree of cellularity of

aging. I was, unfortunately, absent at the time when this question of melanin was raised by Dr. Stern, and I don't know how you settled it. Was it stated that melanin is an expression of the changes that had taken place in aging but is not itself an active component of the cell?

Stern: No.

Oliver: I raised the question as to what melanin is, that is, whether it was the same chemical substance in the brain as in the skin.

Cowdry: My impression is that there are a whole series of melanins

Oliver: Well, I was told by someone that these pigments were essentially identical in the skin and brain.

Stern: Incidentally, I once intended to play around with the dopa reaction in the midbrain, in infantile brains, just before melanin appears, but I mentioned it to a neuropathologist—this was when I was still in London—and he told me he had tried it and didn't get anywhere, so I did not do it. It would be interesting, I think, to attempt it still. But, my main point was that I felt that the fate of melanin in the skin as well as in the central nervous system might be an interesting morphological study from the point of view of aging. I just want to throw it in as a suggestion

Cowdry. Yes, I think it would be a very interesting study

Oliver: I would like to know, if I may persist in my question, whether they are similar things or quite different or only related chemically? Obviously, if it is just a word meaning "black," it means very little

Hamilton: The information that I have on that, Dr. Oliver, is that of John Peters and of Edwards and Duntley (14) at the Massachusetts Institute of Technology. In one case, the approach is chemical, and in the other, spectrophotometric. There are two different kinds of melanin that can be identified spectrophotometrically One form is called melanoid, the other melanin. There is evidently a group of substances, not chemically specific, so that no one particular entity is melanin.

Oliver: Well, one could perhaps call them a family of pigments. Have they many similarities or are they quite different? That is my point.

Hamilton: They are different enough to have different spectrophotometric qualities.

Oliver: They might still have biological similarities

Cowdry: There are certain general similarities, such as cellular arrangement, size of the granules and, in a crude way, the solubility of the granules. They are pigments and they are probably produced by melanoblasts of the same type, but there are superimposed upon these general similarities individual differences. Isn't that what you would say about them?

the tendinous material and the collagenous fibers, too, as representing almost a pure extracellular phase.

Cowdry: There is another claim, that it is intracellular to start with, and becomes extracellular.

Stern: May I ask one more question, please? Does anyone here know whether these cholesterol-like deposits in the skin, as one sees them, for instance, in the bags under the eyes, are a direct reflection of cholesterol level in the blood or atheromatotis or things like that? Is there any direct correlation between cholesterol deposits in the skin and in other tissues?

Oliver: I think it would be generally doubted that there was any direct or simple correlation.

Simms: Are you referring to the *arcus senilis?*

Stern: No, these xanthomatous deposits under the eye.

males and females, seems to me to have considerable significance for an attempt to understand aging That is why I wanted to make sure we don't pass it over. Here we have two organisms (male and female) exposed to much the same environment, maintaining this constant intercourse between the internal and the external environments, mediated through the skin and all the other intakes and outlets: air, water, food, elimination, etc Now, what I am concerned about is this. as we fractionate that organism into different organ systems or different processes and try to find specific factors and variables and so on, are we in danger of forgetting that a dynamic theory of aging has got to consider the total system of the organism that is functioning, and that it is the functioning activities of those two organisms *as a whole* which may be important if we expect to orchestrate the contributions coming from the special factor studies? That is important for a theory of aging

Let me point out my perplexity about it As I understand it, the female at puberty begins to go through a monthly cycle which is not only the hormonal cycle, the gonadal activity, but also the physiological cycle that shows a greater degree of fluctuations than the male Does that indicate that the female organism, as a system, is more elastic or has more dynamic capacity for maintaining equilibrium than the male? If so, that may have a very profound significance on our evaluation of the female reaction to disease, to aging, and so on But then the female apparently loses that flexibility during involution at menopause and yet goes on and lives longer than the male It seems to me that if we begin to think in those dynamic terms, of a system maintained in equilibrium,

the tissues is subject to variation. There are, indeed, so many variable factors, it is extraordinarily difficult to make definite statements.

Oliver: In the discussion of elastic fibers, no one mentioned the lung; only the vessels and the skin have been metioned. There are elastic fibers that can be very nicely studied in the lung, and there is also the advantage that they form a large part of the tissue. One fiber will even be a considerable part of the tissue of a thin alveolar wall. They certainly age and degenerate and rupture.

Cowdry: I think the lung should be investigated, by all means, and by chemical methods, too, to get out the elastin and see whether it is different, because there may be several different elastins. Dr. Jacobson, have you anything to add to this discussion?

Jacobson: Well, I don't think I can contribute anything on the elastic fibers, though I would like to know whether you found any changes in the aldehyde reaction which is given by elastic fibers. You can stain them with Schiff's reagent, and it would be quite interesting to know whether the aged elastic tissue gives a different reaction compared to the young elastic tissues.

Cowdry: I have the feeling that it does, in respect to the Schiff reaction, but I can't put my finger on the evidence just now.

Engle: Well, gentlemen, it is time to adjourn for luncheon. I am very sorry that we didn't get time to pick up this male-selective factor.

Cowdry: Can we have one more idea?

Nelson: I have a question, that's all Do you cytologists consider that collagen fiber is entirely intercellular?

Cowdry: Oh, no.

Nelson: Extracellular? I wondered about Dr Goldzieher's question, in speaking of the intracellular environment's playing a role in the change with age Tendinous material, for example, has a distribution of electrolytes very much the same as the extracellular phase does I should think that the extracellular phase would be the most stable, really, of the internal milieu

Goldzieher: I was talking about the collagenous fibrils, not the elastic fibrils.

Nelson: I see. Well, is that intracellular? I always thought of it as being primarily an extracellular component, being exposed essentially to an extracellular environment.

Goldzieher: The collagenous fiber is intracellular.

Cowdry: Well, there is difference of opinion on that

Goldzieher: There is a difference of opinion, yes, but a considerable body of students claims that their origin is intracellular.

Nelson: It immediately interested me because I always thought of

the tendinous material and the collagenous fibers, too, as representing almost a pure extracellular phase

Cowdry: There is another claim, that it is intracellular to start with, and becomes extracellular.

Stern: May I ask one more question, please? Does anyone here know whether these cholesterol-like deposits in the skin, as one sees them, for instance, in the bags under the eyes, are a direct reflection of cholesterol level in the blood or atheromatotis or things like that? Is there any direct correlation between cholesterol deposits in the skin and in other tissues?

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Simms: Are you referring to the *arcus senilis*?

Stern: No, these xanthomatous deposits under the eye.

Engle: Let's talk about them later. Dr Frank has been trying to get a word in for the last twenty minutes.

Frank: The point Dr. Hamilton raised, about the difference between males and females, seems to me to have considerable significance for an attempt to understand aging. That is why I wanted to make sure we don't pass it over. Here we have two organisms (male and female) exposed to much the same environment, maintaining this constant intercourse between the internal and the external environments, mediated through the skin and all the other intakes and outlets: air, water, food, elimination, etc Now, what I am concerned about is this: as we fractionate that organism into different organ systems or different processes and try to find specific factors and variables and so on, are we *in* danger of forgetting that a dynamic theory of aging has got to consider the total system of the organism that is functioning, and that it is the functioning activities of those two organisms *as a whole* which may be important if we expect to orchestrate the contributions coming from the special factor studies? That is important for a theory of aging.

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a dynamic equilibrium, being self-reparative, then we will not be tempted to look exclusively for one particular factor or variable as important for understanding this system.

I am putting in a plea for consideration of the dynamic aspects of aging and asking what significance comes out of this contrast between female and male, because here we have an experiment. We have two organisms or systems carrying on with the same protoplasm. (That is what I objected to when it was said "different in protoplasm.") The protoplasm is probably the same, but there are two systems that maintain themselves and operate and function in somewhat different ways. Now, can we evaluate those in terms of viability, susceptibility, and aging and so on? If so, then we envisage the problem of aging in larger dimensions.

Engle: I am sure you can't ignore that kind of concept.

Levin: During the discussion a while ago I wanted to call the attention of the group to some work at Columbia about which Dr. J. W. Blunt recently told me. It seems to me their results may have a bearing on the relationship between behavior of the collagenous fibers and the problem of water inhibition in the epidermis, and also, incidentally, this introduces the endocrines again. The Columbia group reported that in studies of healing of experimental wounds made in a standard manner, when they treated the animal with cortisone, the epidermis formed a continuous layer, much as in the normal healing, but the dermis did not, because the collagenous fibers did not form the union as they normally do. The fibers remained there apparently static, the blunt cut ends appearing just as they did at the time of incision, whereas normally the fibers first begin to taper off as though losing a portion of their substance and then start regenerating or being laid down again.

Horvath: That is the same type of repair that is supposed to occur in vitimin C deficiency—superficial and not deep.

Levin: Well, as you are aware, the adrenal cortex is related to vitamin C, too, in a manner of speaking. Maybe this accounts for the similarity.

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have some chemical data, of course, on the composition of the chromatin of a resting nucleus, which is composed of fine threads or chromonemata, the precursors of the chromosomes. In addition to the ordinary chemical analysis of this material, a number of histochemical methods are also available. These methods are summarized in Table I. The Feulgen method will reveal the presence of desoxyribonucleprotein, which we formerly called the thymonucleoproteins. Then we have the methyl green-pyronin method The methyl green will stain the desoxyribonucleoproteins, and the ribonucleoproteins will be stained red by the pyronin However, we found, as I shall show you later, that when both nucleoproteins are present, the red component is not sufficiently strongly absorbed and it requires other methods to reveal, under those circumstances, the presence of ribonucleoproteins. Pyronin alone, in the absence of methyl green, will, of course, pick out ribonucleoproteins wherever they are. Then we happened to find that with an old histological method, the May-Grunwald and Giemsa method, as it has been used by hematologists for many years, the cells will stain differently. The desoxyribonucleoproteins will appear in a purple red, and the

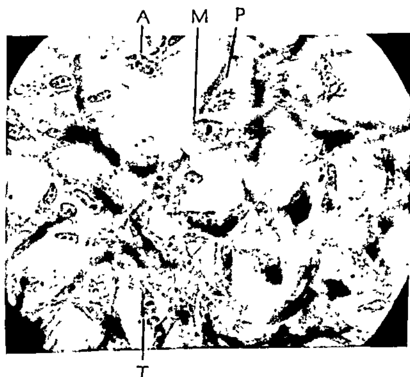


FIGURE 1. Chick embryo osteoblasts grown *in vitro* for 24 or 48 hours May-Grünwald and Giemsa stains P = prophase, M = metaphase; A = anaphase; T = telophase x 300

ribonucleoproteins in a blue color.

I am going to discuss the different stages of mitosis stained by the May-Grunwald and Giemsa method. Figure 1 shows a tissue culture of chick embryo osteoblasts, which we chose to demonstrate the changes in the chromosomes, not because chick embryo osteoblasts show anything in particular but because they present us with material which has a very large number of mitoses in the field A prophase is shown at P in Figure 1 We will see those cells under high power later on. Metaphases are illustrated at M, anaphase at A, and there is one telophase at T. We might also notice that the resting nuclei appear reddish purple The nucleoli are stained dark blue, and the cytoplasm is blue as well, as you can see in cells anywhere in the field.

Figure 2 illustrates these cells under higher power. We can see that the resting nuclei are reddish purple, due to the desoxyribonucleoprotein, and blue stained ribonucleoprotein is found only in the nucleoli Very rarely do we find one or two very fine additional granules of ribonucleoprotein in the nuclear material as in the cell marked R in Figure 3 Otherwise, the nucleus retains nothing but the red purple stain of desoxyribonucleoprotein The cytoplasm of these young growing cells contains large quantities of ribonucleoprotein stained blue Figure 3 shows cells in prophase The nucleolus and the chromosomes which have been formed by the thickening of these extremely fine chromatic strands of the resting nucleus are also demonstrated in Figure 3. These structures can also be seen in the living cell nucleus with phase contrast microscopy as was shown by Fell and Hughes (5). These strands are thickened in early prophase

with the

detail

neces

of the nucleolus Figure 2 also shows cells in metaphase where the chromosomes stain black, since both components, the reddish purple and the blue, are absorbed on the chromosomes in metaphase The chromosomes in prophase stain differently from those in metaphase Prophase and metaphase each require about ten minutes for completion During that time something important has happened to the chromosomes. I want to draw your attention to the fact that the spindle fibers themselves contain no ribonucleoproteins, and the whole area of the spindle in metaphase appears lighter.

Figures 4 and 5 show cells in prophase The cell shown in Figure 4 is about the earliest stage of the prophase you can recognize The extremely delicate chromatin network of chromonemata, in resting nuclei, stains reddish purple In early prophase the strands become thicker. You see that there is hardly any black or bluish stained ma-

FIGURE 4. See legend for Figure 1. x2800



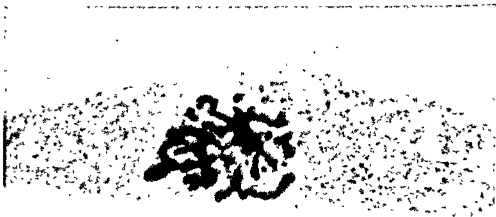


FIGURE 5 See legend for Figure 1 x2800

terial on the chromosomes They still contain practically nothing but the desoxyribonucleoprotein. Nearly all the ribonucleoprotein is gathered in the nucleoli except for five or six minute areas of the chromosome which appear black in Figure 4. The cell shown in Figure 5 is in late prophase. In it the nuclear membrane is just disappearing The chromosomes practically everywhere have synthesized dark blue staining ribonucleoproteins. Thus at the end of prophase, the chromosomes contain both desoxyribonucleoproteins and ribonucleoproteins and appear nearly black. Some short strands of chromosomes are still stained

FIGURE 2. Chick embryo osteoblasts grown *in vitro* for 24 or 48 hours. May-Grünwald and Giemsa stains. P = prophase; M = metaphase, A = anaphase; T = telophase. x425.





FIGURE 3. Chick embryo osteoblasts grown *in vitro* for 24 or 48 hours. May-Grunwald and Giemsa stains. R indicates cell with fine granules of ribonucleoprotein in the nuclear material. x915

Editor's Note. Due to limitations in methods for color reproduction some of the details mentioned in the text and observable in the projection of the original photomicrographs are not clearly demonstrated in the color plates.

FIGURE 4 See legend for Figure 1. x2800.





FIGURE 5 See legend for Figure 1 x2800

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purplish only. These have not formed enough ribonucleoprotein to cover themselves

Returning to Figures 2 and 3 you see a metaphase at M. The chromosomes are black. In anaphase, (Figure 2, A) the chromosomes are of the same coloring, again, but in telophase (T) the chromatin strands are reddish purple again. They have lost the ribonucleoprotein. An anaphase takes about seven to ten minutes, and during this process of the anaphase movement, while the chromosomes move through the cytoplasm of the cell—there is no nuclear membrane here, of course—the chromosomes leave behind them a trail of blue staining ribonucleoprotein which is left in the cytoplasm. When the nuclear membrane is being reconstituted in telophase, this blue material, which has come off the chromosomes and is ribonucleoprotein, stays in the cytoplasm.

Figure 6 is a microphotograph of three anaphases, and the blue material appears black in the figure You see how this trail of blue staining ribonucleoprotein is found in the cytoplasm, while the two sets of chromosomes move apart. The spindle fibers themselves are the lighter strands, and may not be stained by this method They do not contain ribonucleoprotein

A cell in anaphase is shown under high magnification in Figure 7. The dark material is the ribonucleoprotein which has come off the chromosomes, while they moved through the cytoplasm. We assume that it is chromosomal material which has been formed towards the end of prophase while the nuclear membrane was still intact and the nucleolus was still present It has been formed on each of the chromosomes and is now being shed, about fifteen or twenty minutes later, during the anaphase movement In telophase, the chromosomes will contain practically nothing but the desoxyribonucleoprotein They have lost practically all their ribonucleoprotein during the anaphase stages

Figure 2 shows at T two telophases The lower group shows two young daughter cells You can see here already parts of the chromosomal threads, stained reddish purple only, but some parts of the chromosomal threads in this early telophase still contain ribonucleoprotein stained dark blue The same applies to the daughter cell You can see the somewhat coiled strands of chromosomes containing in some areas both blue and reddish purple material Ribonucleoprotein is found nearly exclusively, I should say, in the nucleolus There are very small remnants of blue material in this part of the nucleus, with a few scattered dots In this phase of the cell division, in the daughter cell stage, practically all ribonucleoprotein is now to be found in the nucleoli, with desoxyribonucleoprotein in the chromatin only, which

contains no blue material. The cytoplasm stains blue, as it does in all these young cells, which have a considerable amount of ribonucleoprotein in the cytoplasm. Areas in the cytoplasm of the two daughter cells (Figure 2) contain the material which has just come off the chromosomes, a few minutes earlier.

Now, I want to give you the proof for this statement that the reddish purple material is desoxyribonucleoprotein, and the blue material is ribonucleoprotein. This work was done together with Dr. M. Webb at the Strangeways Research Laboratory (8), and we used four different methods to show that this color scheme holds true. Firstly, we tried the pure substances *in vitro* to see what color reactions we could obtain. Secondly, we used the specific enzymes to digest away either the reddish purple component with desoxyribonuclease or the blue component with ribonuclease. Thirdly, we treated the cells with lanthanum or beryllium ions which form insoluble complexes with desoxy- and with ribonucleoprotein. This made them considerably more resistant to attack by the enzymes. Fourthly, we used specific enzyme inhibitors to prove that the disappearance of this material, when we treated the cells with the enzyme solutions, was really due to the activity of the enzymes themselves.

Table II summarizes the results of these experiments on the substances we tested *in vitro*: thus, desoxyribonucleoprotein derived from herring sperm and desoxyribonucleohistone from calf thymus stain purple red. The free desoxyribonucleic acid without the histone stains bright blue.

Oliver: May I ask just how you do this? Was it a solution of these substances that was tested?

Jacobson: A solution of the isolated substance is put onto a slide, it is dried, and then treated as if it were the cellular material.

The protamine and histone by itself stains pink. If you mix the free desoxynucleic acid with protamine or histone *in vitro*, as we did here, and thus form the desoxyribonucleoprotein complex, you get, again, the purple red color. Similarly, the ribonucleoprotein, for instance pancreas ribonucleoprotein, stains dark blue, whereas the free ribonucleic acid (yeast ribonucleic acid) stains blue; so this method as such, would not allow us to distinguish betwen the ribonucleoprotein and the free ribonucleic acid or the free desoxyribonucleic acid. However, from the digestion experiments (which I will come to presently), we can conclude, and there is other chemical evidence for it, that we don't deal with free desox[illegible]

FIGURE 6 See legend for Figure 1 x 1200.



FIGURE 7. See legend for F

TABLE III

Digestion of Blue Staining Material by Ribonuclease

	Untreated	Treated
Cytoplasm	blue	colorless
Nucleolus	blue	grey
Chromatin	purple-red	purple-red
Chromosomes		
Prophase	purple-red	purple-red
Metaphase	black	purple-red
Anaphase	black	purple-red
Telophase	purple-red	purple-red
Cytoplasm between anaphase chromosomes	blue	colorless

dish purple strands instead of staining black as they do in untreated specimens.

Conversely, if we digest cells with desoxyribonuclease, we find the opposite effect, that is, all the blue material stays untouched in the cytoplasm (Table IV) On the other hand, the resting nuclei are reduced to a faint pink nearly colorless appearance except for the dark blue stained nucleoli. The chromosomes of prophase were colorless, and it was extremely difficult to detect prophases because their chromosomal material had been removed and only very faint bits of chromosomal strands were left Metaphase and anaphase chromosomes were

TABLE IV

Digestion of Purple-Red Staining Material by Desoxyribonuclease

	Untreated	Treated
Cytoplasm	blue	blue
Nucleolus	blue	blue
Chromatin	purple-red	faint pink - colorless
Chromosomes.		
Prophase	purple-red	colorless
Metaphase	black	blue
Anaphase	black	blue
Telophase	purple-red	colorless
Cytoplasm between anaphase chromosomes	blue	blue

TABLE II

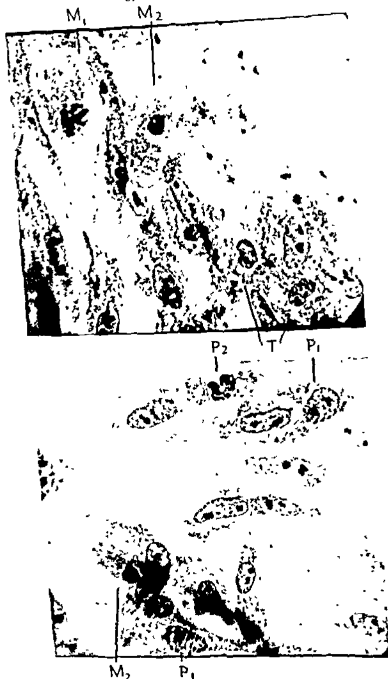
Color Reactions of Desoxyribonucleoprotein and Ribonucleoprotein

Type	Material	Color
DRNP	Herring Sperm Desoxyribonucleoprotamine	purple-red
	Calf Thymus Desoxyribonucleohistone	purple-red
DRNA	Herring Sperm Desoxyribonucleic acid	bright blue
	Calf Thymus Desoxyribonucleic acid	bright blue
Protein	Protamine	pink
	Thymus Histone	pink
DRNA + Protein	Herring Sperm Desoxyribonucleic acid - Histone Complex	purple-red
	Thymus Desoxyribonucleic acid - Protamine Complex	purple-red
RNP	*Pancreas Ribonucleoprotein*	*dark blue*
RNA	*Yeast Ribonucleic Acid*	dark blue

DRNP = Desoxyribonucleoprotein
DRNA = Desoxyribonucleic acid
RNP = Ribonucleoprotein
RNA = Ribonucleic acid

by many investigators previously, showed that the blue material of the cytoplasm was completely removed within thirty minutes (Table III). The blue material of the nucleolus was changed to a colorless gray or a pink-gray color, within thirty minutes The reddish purple desoxyribonucleoprotein-containing material of chromonemata in resting nuclei stayed unchanged in its purple red color. The prophase chromosomes were not changed in any way at all by ribonuclease, which digests away *only the blue material*, whereas metaphase and anaphase chromosomes changed color and appeared purple red, having been deprived by the enzyme of their ribonucleoprotein. In telophase, the chromonemata stayed unchanged *Now, the cytoplasmic* area between the anaphase chromosomes, which contained the blue-staining ribonucleoprotein, which we assume has come off the chromosomes, was colorless. In fact, ten minutes' digestion with ribonuclease removed this material, which seems to be rather labile It is very readily attacked by ribonuclease.

After ribonuclease action the chromosomes have a reddish purple color, whereas the cytoplasm has become quite colorless. The nucleoli disappear from the nuclei, and the chromosomes appear as thin, red-



FIGURES 8 and 9 Chick embryo osteoblasts grown *in vitro* for 24 or 48 hours May Grunwald and Giemsa stains Fifteen minutes after treatment with 1 2000 aminopterin P_1 = early prophase, P_2 = late prophase, M_1 = early metaphase, M_2 = late metaphase, T = telophase x 915

deprived of their purple-red staining material due to digestion by the desoxyribonuclease, and they stained bright blue. The telophase material has also become colorless. The interchromosomal blueness in the cytoplasm due to the ribonucleoprotein, is preserved.

Now, there was one technical difficulty. Desoxyribonuclease has not been used very successfully so far in histochemical investigations because the pure, or so-called purest, desoxyribonuclease contains very small traces of proteolytic enzymes, enough to digest away a quantity of cellular protein within a few minutes The only way we could make the desoxyribonuclease work specifically was by adding an inhibitor of the proteolytic impurity, which is there in infinitesimal traces We found quite effective proteolytic inhibitors in 0 1 molar solutions of cysteine or hydroxylamine Both of these substances completely inhibit the action of the proteolytic impurity, and allowed us to study the action of the desoxyribonuclease only

When tissue cultures are treated with desoxyribonuclease, the chromatin disappears from stained slides. The nuclei appear as punched-out areas but the nucleoli may be seen as blue dots. The background is stained slightly pink but there is nothing of the red purple color present Cells in anaphase often show blue stained material between the two groups of anaphase chromosomes; it stays there practically unaltered, even after forty-five minutes' digestion with the desoxyribonuclease The cytoplasmic blueness is preserved In later-stage telophase, the ribonucleoprotein may remain in the nucleolus unaltered but the chromosomal material disappears There is hardly anything left to prove the presence of prophase chromosomes. They are reduced to ghosts It is impossible to demonstrate them, really.

It is quite an interesting point, that chromosomal ribonucleoprotein goes from the chromosomes into the cytoplasm during the period when the chromosomes are not separated from the cytoplasm by a nuclear membrane The function of this material is not known, but I think it is certainly worth while to investigate what it does. We studied it only in cells which show a fairly good growth rate, for practical reasons. For instance, we use chick embryo osteoblasts or fibroblasts. We also studied it in bone marrow cells, from animal and human material. We saw it in intestinal epithelial cells, but it is quite possible that this is still a characteristic of fast growing cells only, and it is possible that cells which have a much slower growth rate or cells which no longer divide, after this division, may not show the shedding of ribonucleoprotein from the anaphase chromosomes. I think this phenomenon must have some meaning What its meaning is, of course, is difficult to say



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Frank: Do you know the pH of this substance?

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Figure 9, P_1 illustrates a very early prophase where a few strands of the reddish purple chromosomes are already stained bluish black due to the ribonucleoprotein synthesized by them; so this process is not affected by the folic acid antagonists. A later prophase is shown at P_2 (Figure 9) and a metaphase with clumped chromosomes at M. This clumping of chromosomes is also shown at M in Figure 10. This is not a pycnotic nucleus. It is a cluster of tightly packed metaphase chromosomes At M (Figure 10), is a metaphase which was caught just at the moment when the folic acid antagonist was dropped on the cells. A group of chromosomes have slipped away but the bulk of them got stuck in the center of the cell.

A mitotic count on this material shows that the distribution of the different phases of mitosis is affected in the following way. whereas in the control cultures, about 20 per cent of the dividing cells are in prophase, 35 per cent are in metaphase, and roughly 20 to 25 per cent are in anaphase and in telophase (Figure 11). Fifteen minutes after aminopterin 1·2000 has been dropped onto these cells in order to replace as much of the folic acid or its derivitives as we possibly can, we find that there is just over 30 per cent of the cells in prophase, nearly 60 per cent have been able to proceed up to metaphase, but none of the cells was able to perform an anaphase movement, and those cells which were in anaphase went into telophase It is the step from meta- to anaphase which has been blocked by aminopterin This compound prevents folic acid or its derivatives from functioning in the cells. In other words, we conclude that folic acid, or a substance very closely related to folic acid, is actually functioning during metaphase and throughout anaphase.

Cowdry. Does colchicine do the same thing?

Jacobson. Colchicine will do the same The only difference is that with colchicine we have no idea what it blocks, whereas with folic acid antagonists we can assume that they replace either the folic acid molecule or a very similar compound The folic acid antagonists give us information about physiologically functioning molecules in the dividing cells

Heilbrunn: How can you assume it has no other effect? You are using it in fairly high concentration.

We are very much interested in the mode of action of folic acid antagonists on the cells. The reason for that is twofold. Folic acid is a growth factor and folic acid antagonists are, perhaps, the most effective means of prolonging the life span of cases of acute leukemia. We wanted to know how these substances act, so we studied their effect on cells grown *in vitro* as well as on normal and leukemic bone marrow cells, and I feel that there is perhaps, some small justification for putting these data before you, because cell growth, I think, is intimately connected with the phenomenon of life, and, as such, is intimately connected with the phenomenon of aging.

Folic acid is a factor which plays an active role during the brief period of mitosis. The action of folic acid antagonists on cells is of complex nature. If you drop onto a tissue culture a folic acid antagonist such as aminopterin which blocks the availability of folic acid and its derivatives, you will find that within fifteen minutes the following changes have taken place (Figures 8, 9, 10): a) The resting cells are hardly affected. I am not sure whether the nucleoli are really larger, but I don't think there is very much change in the resting cells. b) The prophases occur fairly normally, although perhaps they are not quite normal c) There is interference in passing through metaphase Figure 8, M shows an early metaphase. Later metaphase is hardly recog-

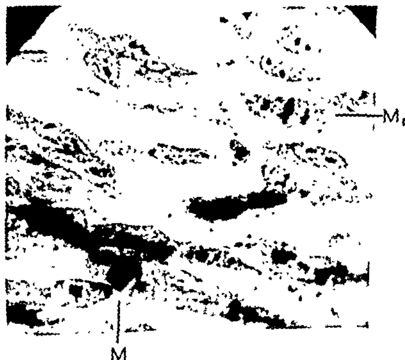


FIGURE 10. See legend for Figures 8 and 9. x 915

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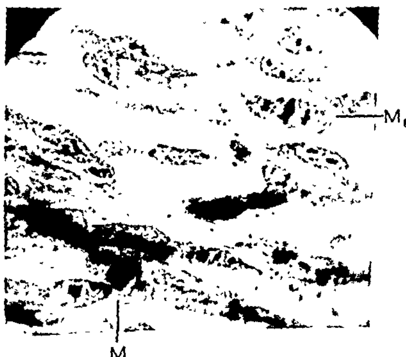


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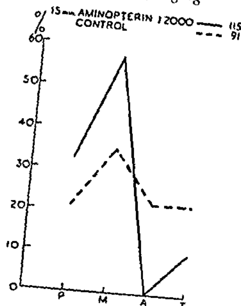


FIGURE 11 Percentage distribution of cells in prophase (P), metaphase (M), anaphase (A), and telophase (T) in chick embryo osteoblasts grown *in vitro* for 24 or 48 hours

------- Control cultures

——— 15 minutes after treatment with 1 2,000 aminopterin

Figures in the right upper corner give the average number of cells in division per tissue culture

Jacobson. This is a very high concentration in order to replace in the cell every amount of folic acid available as quickly as possible.

Heilbrunn: But isn't it assumed that it has no other effect than to antagonize folic acid?

Jacobson: Well, it replaces either folic acid or possibly it interferes with a close derivative of folic acid, folinic acid, and you can counteract this inhibitory effect by the *Leuconostoc-citrovorum* factor which is another name for folinic acid

Heilbrunn: I may be slow to understand, but do you mean a substance may certainly be an antagonist of folic acid and may have other effects as well?

Jacobson: You can reverse the toxic effect of aminopterin with folinic acid or *Leuconostoc-citrovorum* factor The *Leuconostoc-citrovorum* factor seems to be the substance into which folic acid is converted in the cell; so if you replace folic acid or antagonize the availability of folic acid by the folic acid antagonist and then give the end-product of this process, namely folinic acid, so that the cell is no longer required to convert folic acid into this factor, you can completely counteract the action of aminopterin or any of the folic acid antagonists.

Levin: In your particular system here?

Jacobson: We haven't tried folinic acid on this type of culture yet. We know it from other experiments, because the *citrovorum* factor was only synthesized after my arrival in this country.

Leuin: Yes, I asked that because this is the experiment I think you would have to perform in order to answer Dr. Heilbrunn's question.

Jacobson. The experiment has been performed by others in a more general way, by giving aminopterin to animals in such quantities that the animal would have died within a few days. When given the *Leuconostoc-citrovorum* factor simultaneously, the animal not only develops normally but no tissue change is observed and its growth is even enhanced (2, 3, 10).

Heilbrunn: What interests me is that in tissue culture the effect is so immediate, whereas, the effects on animals require days.

Jacobson. That is because you can't give 1 2000 aminopterin to an animal

Heilbrunn· Do you think you need eventually to do the experiment directly?

Jacobson: As soon as I am back in Cambridge I will try the effect of simultaneous treatment with aminopterin and the *Leuconostoc-citrovorum* factor on tissue cultures

Engle Dr Jacobson, what information do you have as to the role of oxygenation at this point? This action occurs in terms of seconds—does it interfere with oxygen consumption?

Jacobson· No If you poison the cells with cyanide, it affects the mitosis very little During the actual mitosis, during the formation and movement of chromosomes,—and here I could quote experiments by Dr Hughes (6)—the oxygen uptake is not essential for the cell in this system, for this effect

Heilbrunn. That is true of frog eggs, too

Jacobson This effect, of course, comes from [illegible] directly [illegible]

in [illegible] I can judge We don't make stained preparations but we do study the protoplasmic viscosity The polysaccharides prevent mitosis, and they have no relation to folic acid

Jacobson· Oh, you can stop mitosis in many ways The reason why I report this is that the effect of aminopterin reveals to us the part that folic acid plays in this process I am not *reporting* this to show that we can stop mitosis This is really used as a tool to show that there is a substance normally functioning in the mitosis of every cell we have tested and that substance is folic acid or a close derivative of folic acid, like the *Leuconostoc-citrovorum* factor

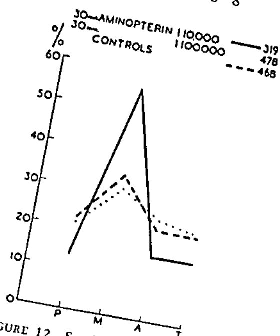


FIGURE 12. See legend for Figure 11.

······ Control cultures.

—— 30 minutes after treatment with 1:10,000 aminopterin.

- - - 30 minutes after treatment with 1:100,000 aminopterin.

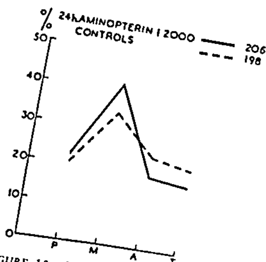


FIGURE 13. See legend for Figure 11.

······ Control cultures

—— 24 hours after treatment with 1:2,000 aminopterin

Heilbrunn: In order to prove that, you would have to show that this concentrated solution of aminopterin has no other effect than its effect on folic acid

Jacobson: Or the *Leuconostoc-citrovorum* factor—

Heilbrunn: Yes, or that

Jacobson: —which is a derivative of folic acid.

Heilbrunn: And that would require real proof

Jacobson: Well, let me go on with the story, to show what other effects we have observed

For instance, if these tissue cultures are observed about thirty minutes after a 1 10,000 solution of aminopterin has been dropped on the cells, the effect of the aminopterin is very much less marked (Figure 12). Already a few cells go through the anaphase stage. However, twenty-four hours later, in 1:2000 concentrations of aminopterin, the cells divide nearly normally (Figure 13).

A-methopterin, which is a folic acid antagonist in which two molecular groupings are substituted, so it has two points in the molecule where it is different from folic acid, in concentrations of 1:400, is not as effective an inhibitor as is 1.2000 aminopterin. The most powerful inhibitor for this system is aminopterin, as we know already from clinical and other experimental work. In the less powerful antagonist, A-methopterin, two groups are substituted in the folic acid molecule, the hydroxyl group in position four and the hydrogen atom in position ten of the molecular formula

Twenty-four hours after the addition of aminopterin in concentrations of 1 2000, there is hardly a difference between the control and the treated cultures, in other words, the fibroblasts or osteoblasts are capable of completely overcoming the inhibitory action of aminopterin, in contrast to other cells which could be irreversibly damaged. It is very interesting that another antagonist, A-methopterin, which has in addition to the 4-amino group in the aminopterin [illegible]

[illegible] graph (Figure 14) represents cell counts of the control cultures. This antagonist of folic acid, A-methopterin, which was initially very much less [illegible]

prolonged inhibitory

Similar changes we [illegible] if you give, for instance, a leukemic mouse, 1 milligram of aminopterin and kill the animal two hours later, then you will find there are still a number of cells in prophase, with their purple-red stained chromosomes (Figure 15). At M_1 of this figure an abnormal metaphase is shown. At M there are two metaphases, where the chromosomes are clumped together

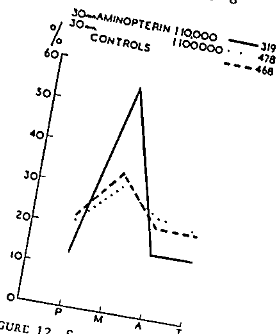


FIGURE 12 See legend for Figure 11

- - - - - - Control cultures
———— 30 minutes after treatment with 1 10,000 aminopterin
· · · 30 minutes after treatment with 1 100,000 aminopterin

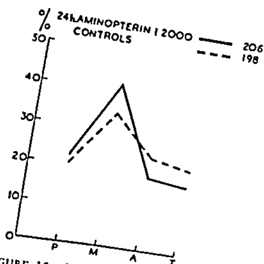
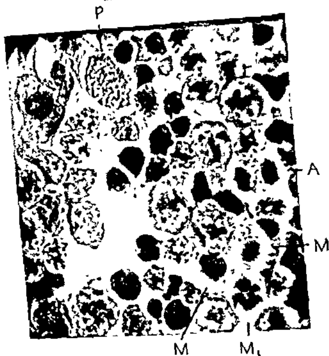
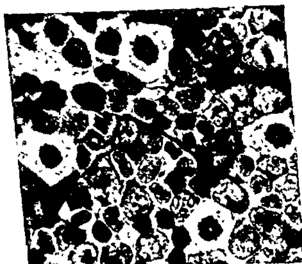


FIGURE 13 See legend for Figure 11.

- - - - - - Control cultures
———— 24 hours after treatment with 1 2,000 aminopterin





FIGURES 15 and 16 Smear of bone marrow of leukemic mouse 2 hours after subcutaneous administration of 1 mg aminopterin May-Grunwald and Giemsa stain x 915 P = prophase, M and M_1 = metaphase, A = anaphase of lymphoblasts or stem cells Figure 15 shows three metaphases.

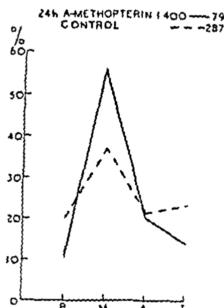
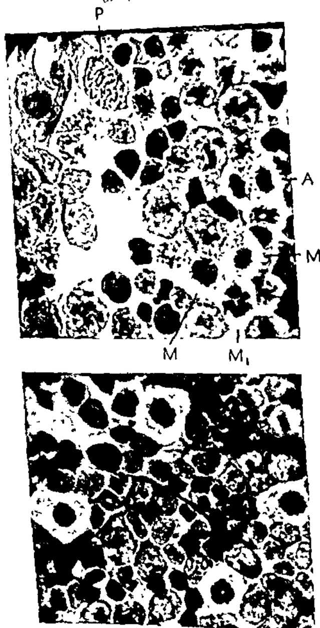


FIGURE 14. See legend for Figure 11
------ Control cultures
——— 24 hours after treatment with 1,400 A-methopterin

and at A there is an anaphase, a stage which has become very rare indeed.

In the intact animal, we see the same effect as in explants In Figure 16 there are four metaphases in a single field of bone marrow taken two hours after a milligram of aminopterin had been given subcutaneously to a leukemic mouse Mitotic counts made on such material demonstrate the following changes; arrest in metaphase and very few anaphases (Figure 17).

In control bone marrow, we find that out of all dividing cells, some 20 per cent are in prophase, over 40 per cent in metaphase, 20 per cent in anaphase, and about 10 per cent in telophase Two hours after aminopterin has been given, we find an increase in metaphases, and very few cells have proceeded through anaphase into telophase The data shown in Figure 17 are based on 16,000 cells counted We find that out of all nucleated cells, 0.63 per cent were in mitosis in the untreated leukemic bone marrow, and 1 79 per cent in the aminopterin-treated leukemic bone marrow. The reason for this apparent increase is that a mitosis takes about forty minutes and 3 times 40 minutes is about 120 minutes or two hours, and we find that 1 89 per cent corresponds approximately to 0 63 per cent multiplied by 3 In other words, the cells have entered into mitosis at about the same rate during the two hours after the injection of aminopterin as they would have done normally, but most of them are stopped in metaphase by the action of the folic acid antagonist.



FIGURES 15 and 16 Smear of bone marrow of leukemic mouse 2 hours after subcutaneous administration of 1 mg aminopterin May-Grunwald and Giemsa stain × 915 P = prophase, M and M_1 = metaphase, A = anaphase of lymphoblasts or stem cells. Figure 15 shows three metaphases.

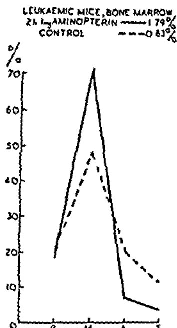


FIGURE 17. Percentage distribution of cells in prophase (P), metaphase (M), anaphase (A), and telophase (T) in bone marrow from leukemic mice.

——— Two hours after subcutaneous administration of 1 mg. aminopterin
- - - - Untreated leukemic animals

Data based on 16,000 cells counted. Figures in the right upper corner give the percentage of nucleated cells dividing

Similar results are found in human leukemia. Eventually in human bone marrow, you find that all dividing cells can be wiped out if you are not careful with folic acid antagonists, or the bone marrow may show severe megaloblastic changes Apart from the plasma cells which have a very low rate of division indeed, and thus survive, and the reticulum cells which have also a very low rate of mitosis, only a few scattered cells may be left over and the bone marrow eventually becomes severely depleted. The dividing cell, therefore, is affected by these types of compounds.

In contrast, if you give folic acid antagonists in very low concentrations, such as usually are employed in human therapy, mitosis may proceed more normally in bone marrow

We have now investigated, with Dr Farber in the Children's Hospital in Boston, a large number of bone marrows from patients treated with folic acid antagonists. We find exactly the same effect, provided that enough material has been given. There is a very marked accumulation of cells in metaphase, and, in fact, we find in some bone marrows between three and eight times as many metaphases as anaphases If we were to draw the curve of the distribution of the different stages

of mitosis, the prophases would be about 20 to 25 per cent, the metaphase about 60 to 65 per cent, with less than 10 per cent anaphases and telophases. A very characteristic pattern is obtained by the action of folic acid antagonists.

Engle: Dr Jacobson, I take it from your remarks that you feel all of these cells which are arrested at metaphase are arrested only very temporarily, and, in a matter of some minutes, they will go ahead and complete their division.

Jacobson: No, sir. I think, for instance, in the bone marrow, many cells will be unable to overcome the inhibition with folic acid antagonist, and they will eventually die and disappear. That must be the effect of the therapy because we find in about 30 to 40 per cent of these cases, eventually all the fast-dividing leukemic cells have been wiped out. In contrast to this, for instance, the fibroblasts have mechanisms in their cytoplasm by means of which they can overcome this antagonist. You see, the antagonist aminopterin differs from the molecule it antagonizes by one amino group, and it is quite feasible that the cytoplasm has enzymes to deaminate this material. However, not all cells contain it, so that is why we originally thought that folic acid was only required in the life of certain types of cells, because we couldn't show an effect with folic acid antagonists on many cell types. But if you look at the cells immediately after you apply the antagonist, you see quite a dramatic effect on every dividing cell, before they have time to convert the antagonist into something else.

Engle Well, then, in your osteocytes, you are developing embryonic bone cells What would be your estimate of the length of time required for this cell nucleus to deaminize the aminopterin?

Jacobson: To overcome, for instance, the aminopterin effect, I should say might require anything between two and six hours

Engle: Oh, so long?

Jacobson. Yes I have observed, after one hour, there may still be a marked effect, but after twenty-four hours, there is none at all. With another antagonist, A-methopterin, which cannot be so readily converted into an indifferent substance, as can aminopterin, there was a lasting effect, and even after twenty-four hours, there was a considerable arrest in metaphase, and many cells never reached that stage where they could divide again, because they degenerated in metaphase.

Leim: In the case of the A-methopterin you started with a higher concentration I believe you said five times as great as of the aminopterin

Jacobson: Yes, initially the concentration of 1·400 A-methopterin did not do as much as 1:2000 aminopterin, so it is a less powerful in-

FIGURE 18. Normal small intestine of the mouse. Haematoxylyn-eosin. x 135.





FIGURE 19. Small intestine of the mouse 3 days after subcutaneous injection of 0.2 mg aminopterin Haematoxylyn-eosin x 135

hibitor per molecule, but it is a much more lasting inhibitor in these cells.

Leim: What you are actually postulating, then, is that the fibroblasts have a better deaminating mechanism than does the animal *in toto*. This is a little surprising to me. I would expect the intact animal which possesses its liver, kidneys, etc. to be rather good at deaminization.

Jacobson: Yes The point is that some cells don't seem to have this property at all. Once the aminopterin molecule has replaced the folic acid molecule in these cells it stays there. That is why we find the great sensitivity to folic acid antagonists in bone marrow and also, surprisingly enough, in the intestinal epithelial cells, as I will show you.

Figure 18 shows a normal mouse intestine, and I want you to notice that the glands are quite normal, short ones There are about a hundred epithelial cells required to cover a villus, or something of that order. If you give a mouse 0 2 mg of aminopterin, three or four days later, the intestine shows a dramatic change (Figure 19). The villi are now maximally contracted because there are only about ten cells available to cover the villus and each of the epithelial cells is extraordinarily large and swollen You see, in the stroma, how the whole villus is contracted The epithelial cells covering the villi are enormous in size. The glands are lined by an epithelium which shows considerable pleomorphism, thin cells and large ones, and, under high power, we find these large swollen epithelial cells (Figure 20) There are also others, very thin and elongated, which line the gland (Figure 20, E). Figure 21 shows at M a pycnotic cluster of chromosomes in metaphase. It is not a lymphocyte nucleus One can see it is really composed of very tightly packed metaphase chromosomes

In Figure 21 these large nuclei are well illustrated. At E of this figure there are very large epithelial cells The very large swollen cells will degenerate in another day.

In Figure 22, A illustrates one of the few anaphases we have found In the cytoplasm between the two groups of chromosomes, dark stained basophilic material may be seen which has come off the chromosomes At E in Figure 22 are these large, swollen nuclei. I show this because folic acid or its derivatives is required by the dividing intestinal epithelial cells as by other dividing cells If these substances are replaced by aminopterin, we find very severe disturbances * The same changes may occur in the human intestine.

Figure 23 shows the intestine about three or four days after the

* Dustin (4) also observed mitotic changes in the intestinal glands after injection of folic acid antagonists

FIGURE 20. See legend for Figure 19. Note the variation in the size of epithelial cells covering the villi and lining the glands. x 400.



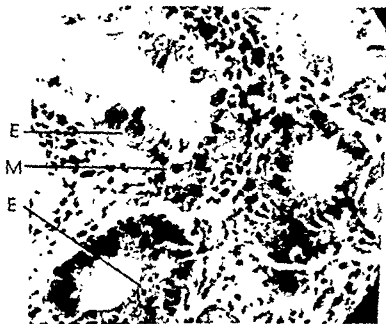


FIGURE 21. See legend for Figure 19. Note large epithelial cells in the glands (E) and an arrested *metaphase* with clumped *chromosomes* (M) x 400

FIGURE 22. See legend for Figure 19 A = anaphase, E = some of the large epithelial cells lining the glands. May-Grünwald and Giemsa stains. x 400.



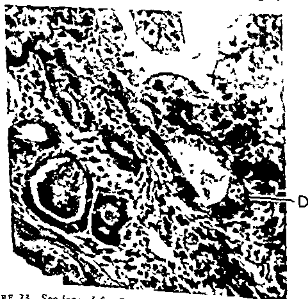


FIGURE 23 See legend for Figure 19 Groups of degenerated cells in the glandular epithelium are shown x 400.

FIGURE 20. See legend for Figure 19. Note the variation in the size of epithelial cells covering the villi and lining the glands. x 400.



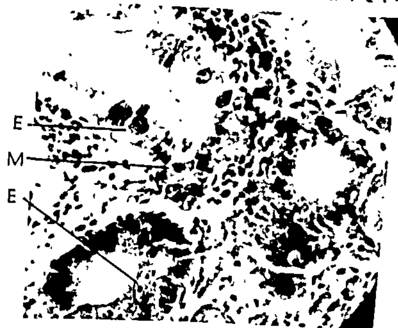


FIGURE 21. See legend for Figure 19. Note large epithelial cells in the glands (E) and an arrested metaphase with clumped chromosomes (M) x 400.

have degenerated completely. The mice grow and are healthy, and you can go on giving them aminopterin, provided you give at the same time sufficient amounts of either folic acid or the *Leuconostoc-citrovorum* factor. The *Leuconostoc-citrovorum* factor can neutralize aminopterin, according to the work of Jukes (2) and others (3, 10) in the ratio of about 1 to 1. But if you work with such highly lethal concentrations of folic acid antagonists and you try to counteract the effect of aminopterin with folic acid itself you have to give about a hundred times more or even four hundred times more, to prevent the blocking effect of aminopterin

Cowdry: In other words, if you stain intestinal mucosa for basement membrane, would you find the basement membrane intact all around?

Jacobson: Well, not intact at the tip of the villi because there are already, at this stage, some destructive processes going on, although the animal was sacrificed before it died of septicemia. But near the base of the villi, you would be able to find the reticular fibers of the basement membrane intact, particularly down below in the glands.

Cowdry: The basement membrane would be required for regeneration, wouldn't it?

Jacobson: Yes, but at this stage there is no epithelium left on the villi The whole of the epithelium is gone We have seen the same in humans, too There are patches in some areas where a few cystic glands are still left, in which we could visualize that regeneration could occur, if the condition were compatible with life. But once it has reached this stage, there are really inches of intestine where there isn't a single epithelial cell left any more Of course, the life span of the epithelial cells of the villi seems to be of the order of about four to five days and then, after that time, if they are not replaced, the animal dies with a bacteremia

Now, as the point has been raised of how far folic acid is being replaced by folic acid antagonists, I might mention the relation of this factor, folic acid, to the *Leuconostoc-citrovorum* factor, as far as we know it nowadays It seems to have a very interesting relation to folic acid But if you would rather not—

Engle: No, please do.

Jacobson. There does not seem to be a dividing cell which doesn't require folic acid, or the derivative of folic acid, the *Leuconostoc-citrovorum* factor It is called the *Leuconostoc-citrovorum* factor because it was discovered over two years ago in Madison as an essential substance in the growth of this nonpathogenic streptococcus. Sauberlich and Baumann (12) noticed that liver extract or yeast contained a substance which promoted the growth of this streptococcus The



FIGURE 24 Small intestine of the mouse 5 days after subcutaneous injection of 0 2 mg aminopterin. Haematoxlyn-eosin Upper parts of the villi (V) denuded of epithelium and covered by a fibrinous membrane with bacteria and cell debris. x 400

injection of aminopterin The breaking down of the epithelium, the process of degeneration (D), pycnotic nuclei, and generating cells are well illustrated. There is still a marked pleomorphism in the glandular epithelium Subsequently, the whole epithelium breaks down (Figure 24). This is not a post-mortem change of the intestine The villi (V) become completely denuded of epithelium and are coated with a fibrinous membrane with bacterial and cellular debris. The animal will die of bacteremia, the whole epithelium having degenerated within three to five days.

Engle: Dr. Jacobson, I have seen precisely this type of change in the intestinal mucosa of mice two or three days after they have been given radioactive substances.

Jacobson: Yes, you can produce somewhat similar changes with x-rays or nitrogen mustard or folic acid antagonists What I find so interesting is that with the folic acid antagonist, which is 4-amino folic acid, with an amino instead of an hydroxyl group in position four on the pteridin ring, you can produce this You can counteract this effect with a derivative of folic acid, tetrahydroformyl folic acid (folinic acid). That has been done on the cells shown You are getting these changes which may be reversible unless, of course, the epithelial cells

on chromosomes, without using folic acid or the *citrovorum* factor, but for the coming off of ribonucleoprotein from the chromosomes or the shedding of ribonucleoprotein into the cytoplasm, this molecule, folic acid or the *Leuconostoc-citrovorum* factor, seems to be essential.

Heilbrunn: We have been studying marine egg cells and came to the conclusion years ago that the actual mechanism of cell division was not necessarily tied to the nucleus at all, because it is quite possible to get cells to divide without a nucleus. You said this was necessary for all cells, and I was wondering how inclusive that statement was meant to be For instance, would it apply to the root of an onion?

Jacobson: This material, I should say, precisely, was required for all cells *tested*.

Heilbrunn: Does that include plant cells, root cells? Marine egg cells?

Jacobson: It certainly is essential for amphibian cells, as far as it has been tested; for avian cells; and for mammalian normal and tumor cells, as far as they have been checked. We haven't found any dividing cell that we could lay hands on and on which we could drop aminopterin which did not show this effect.

Heilbrunn Yes, but when you use aminopterin in one part in two thousand, you are really using a concentrated mixture. In some of these experiments we do, we use substances in concentrations of 10^{-6} molar.

Jacobson: Dr. Heilbrunn, what we want to achieve with this concentration is to replace the folic acid or folinic acid at the site where it functions—during anaphase, a process lasting anywhere between seven and ten minutes If you use lower concentrations, for example, 1 mg per 100 g mouse, you get the effect which we showed in the bone marrow Two hours later you find this enormous delay, but not the *complete* interruption of the anaphase process

Heilbrunn: My own feeling is this: I have seen a good deal of research on mitosis, and over and over again people have been strongly stimulated by the advances in organic chemistry and have found that some molecular configuration is necessary to incite cells to divide, and that some slight deviation from that molecular configuration will prevent the action, and then, when more and more research has been done, it has been found that these particular molecular configurations are not so specific as was once thought, so that it goes a little against the grain—it is a matter of prejudice, possibly—to conceive of a particular and peculiar molecular configuration as essential for mitosis

Jacobson: But you may regard it as a prosthetic group of an enzyme which is working in the cell and not as a nonspecific chemical substance Here, we may be dealing with a prosthetic group to an enzyme which achieves something.

growth-promoting action could not be replaced by folic acid itself or by vitamin B_{12}, nor by any other substance. But they noticed also that human urine contains some of this growth-promoting factor, and when they administered folic acid to humans the amount of the *Leuconostoc-citrovorum* factor excreted in the urine was considerably increased (11). This experiment proved that the factor must be closely related to folic acid; otherwise, the human, fed quantities of folic acid couldn't excrete more of this material. It has now been synthesized, and I will discuss its structure as far as we now know it.

The pteridyl ring is present, just as in folic acid. The pteridyl ring is very closely related to the purine ring. The one difference is that two nitrogens are linked by one carbon in the purine ring but in the pteridyl ring they are linked by two carbons, and carbon atom four carries an hydroxyl group and carbon atom two carries an amino group.

Heilbrunn: Are these substances normally acid unless you neutralize them?

Jacobson: Yes. Part of the molecule contains glutamic acid and another part contains folic acid.

Heilbrunn: And is the antagonist also an acid?

Jacobson: Yes. The antagonist which I described was aminopterin, which carries, in the four position, an amino group instead of the hydroxyl group. The other antagonist, called A-methopterin, carries at the ten position a methyl group in addition to the amino group in position four.

Now, let's go back to the conversion of folic acid to the *Leuconostoc-citrovorum* factor, which requires two steps The nitrogen numbered ten carries a formic acid residue, instead of hydrogen as in folic acid. Thus, there is a formyl group there, and then some of the double bonds in the pterin ring are replaced by hydrogen, so it is a tetrahydro-formyl folic acid. It is not quite certain at which point the hydrogenation of the pteridyl ring takes place

It is very interesting that an enzyme, like xanthine oxidase, can reduce the pteridyl ring, but I don't want to go into that now (7). The functioning molecule in the cell seems to be the *citrovorum* factor or folinic acid, as it has been called by Shive (13), who synthesized it. Dr. Parker and his colleagues (1) of the American Cyanamid Company have also synthesized the *citrovorum* factor. It is interesting to think that this material is not required for the cell to enter into mitosis and to proceed up to metaphase. The cell can do that without folic acid or *citrovorum* factor being available. But during the anaphase movement, this type of molecule is required That means, in other words, that the cells are capable of synthesizing ribonucleoprotein, even

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Jacobson: Yes. Part of the molecule contains glutamic acid and another part contains folic acid.

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Jacobson: Yes. The antagonist which I described was aminopterin, which carries, in the four position, an amino group instead of the hydroxyl group. The other antagonist, called A-methopterin, carries at the ten position a methyl group in addition to the amino group in position four

Now, let's go back to the conversion of folic acid to the *Leuconostoc-citrovorum* factor, which requires two steps. The nitrogen numbered ten carries a formic acid residue, instead of hydrogen as in folic acid. Thus, there is a formyl group there, and then some of the double bonds in the pterin ring are replaced by hydrogen, so it is a tetrahydro-formyl folic acid It is not quite certain at which point the hydrogenation of the pteridyl ring takes place

It is very interesting that an enzyme, like xanthine oxidase, can reduce the pteridyl ring, but I don't want to go into that now (7). The functioning molecule in the cell seems to be the *citrovorum* factor or folinic acid, as it has been called by Shive (13), who synthesized it. Dr. Parker and his colleagues (1) of the American Cyanamid Company have also synthesized the *citrovorum* factor. It is interesting to think that this material is not required for the cell to *enter* into mitosis and to proceed up to metaphase. The cell can do that without folic acid or *citrovorum* factor being available But during the anaphase movement, this type of molecule is required. That means, in other words, that the cells are capable of synthesizing ribonucleoprotein, even

Oliver: I didn't appreciate the fact that the nucleoli persisted, or that the accumulation occurred while the nuclear membrane was intact.

Engle: Dr. Jacobson, I should like to ask whether you have investigated the effects of aminopterin on dividing cells of the testis tubules.

Jacobson: No, we have not investigated the gonads at all as yet. That is being done by somebody else.

Engle: I am disappointed. That is the first thing I would have looked at. Then you wouldn't be able to give me any information at all as to whether the same process was true of meiosis, for instance, as it is in somatic mitosis?

Jacobson: I should have made it quite clear right at the beginning, that what I talked about was the somatic mitosis and not meiosis. I am sorry I forgot about that.

Heilbrunn: Can you buy this material, aminopterin?

Jacobson. Oh, yes. Even more, if you want it, I should be delighted to give you some

Heilbrunn: I should very much like to try it.

Hamilton: What is the minimum dose, or is there any minimum dose, of aminopterin or any of the other antagonists that can be given periodically and in nonlethal fashion to the whole animal?

Jacobson: That is being worked out. In the human, for instance, you run into difficulties if you give a milligram per day. In a mouse, I think you have to give about 002 milligrams per day, and there you might run into difficulties after about three weeks

Engle Dr Jacobson, won't you please sit down and be comfortable with the rest of us? In this country, one of the first bits of work that brought aminopterin to my attention was the work of Roy Hertz in Washington, who, using the oviduct of the chick, indicated that aminopterin served as the estrogen inhibitor, and went along with further analysis [illegible]

tract [illegible]

give [illegible] Would you have any further comments to make on that in the light of your general experience?

Jacobson Yes, I saw some of the lantern slides, which Dr Hertz used to illustrate his talk, and I was struck by the number of cells in metaphase Again cells were hit at the moment when they had to proliferate and while they were dividing I think he mentioned at the time that mitosis does not seem to be affected pointing out the numer- [illegible] it doesn't mean that [illegible], some of the tissues [illegible] percentage in the number of mitoses, but very few

Heilbrunn: It achieves it by relation to the chromatin in the nucleus, you think?

Jacobson: It has something to do with the coming off of ribonucleoprotein from the chromosomes during anaphase, and the movement of this material into the cytoplasm.

Heilbrunn: And yet, the chromosomes are not necessary for division?

Jacobson: Well, there is no mammalian cell and no avian cell and no amphibian cell, I think, that divides normally unless it has a nucleus.

Heilbrunn: It was shown first for the salamander egg, I believe, that it could divide without a nucleus.

Jacobson: Cytoplasm of an egg cell can undergo "segmentation," but not the somatic cell.

Stern: Is the chemical constitution of colchicine known?

Jacobson: Not absolutely accurately. It is still under discussion As far as the molecular structure of it has been suggested, it does not allow you to draw any conclusions as to what chemical process is interrupted by colchicine.

Heilbrunn: We have some very clear evidence on colchicine Before the spindle forms, there is a gelation, which I call the mitotic gelation Colchicine has been shown by Beams and Evans, and also by a student of mine, Karl Wilbur, to prevent gelation. Under those conditions, there is no mitotic spindle formation.

Stern: I was just wondering whether colchicine is chemically related to aminopterin

Jacobson: Not at all, as far as we know

Oliver You used the term, "synthesis," in speaking of the accumulation of this ribonucleoprotein. Do you mean that literally? Why couldn't it be an absorption from the cytoplasm or from the nucleoli?

Jacobson: Well, the nucleoli are still present when this material appears on the chromosomes during prophase and it doesn't appear on the chromosomes in continuity with the nucleolus.

Oliver: That is ruled out, then.

Jacobson: During early prophase some chromosomes contain a bit of ribonucleoprotein, quite like a little island of ribonucleoprotein away from the nucleolus It appears on the chromosomes before the nuclear membrane disappears. Now, ribonucleoprotein is a very large molecule, and it would be difficult to think that this could diffuse through the nuclear membrane. We can't be absolutely sure about it, but I don't think we have any evidence to suggest that it is either the mere absorption of cytoplastic nucleoprotein, or that it is all derived from the nucleolus. Actually, there must be some new synthesis sometimes, because—

this mechanism is exhausted the cell stops dividing.

Coudry: May I state that a little differently? Would it be safe to say that the end of the sequence of intermitotic life is terminated that way?

Jacobson: Yes, I think that sounds to me like an extremely important point. I can't answer it, though.

Oliver: I wanted to ask, is it implied that the action of the aminopterin is to interfere with the synthesis or the accumulation of the ribonucleic acid and that this is the mechanism of the disturbance to mitosis?

Jacobson: Well, I should say that the synthesis of ribonucleoprotein on the chromosomes does not seem to require folic acid, because aminopterin does not interfere with that process But I think it has something to do with the catabolism of ribonucleoprotein. Let me give you another example for this. If you have a patient, for instance, who suffers from folic acid deficiency and has, on the basis of the folic acid deficiency, a megaloblastic bone marrow, you find that the cytoplasm of the early megaloblast contains more ribonucleoprotein, more blue-staining basophilic material, than the normal bone marrow. The nuclei are larger, so the shortage of folic acid has not interfered with the increase of desoxyribonucleoprotein It has not interfered with the synthesis of cytoplasm nucleoprotein But it has interfered with the catabolism of the nucleoproteins If you give to such patients 75 milligrams of folic acid, within eight hours the cytoplasmic basophilia of many megaloblasts has disappeared, to such an extent that it would be difficult to show a true megaloblastic picture. The cell can synthesize nucleoprotein, ribo- and desoxyribonucleoprotein, without necessarily requiring folic acid or its derivative, the *Leuconostoc-citrovorum* factor But the catabolism of nucleoprotein certainly requires folic acid or the *Leuconostoc-citrovorum* factor

Hamilton I am still impressed with the action of aminopterin directly on the cell, and I am wondering if there is any way to promote such an effect in a long-lasting fashion by topical administration Again, I am back to skin. Is there any way that some of these antagonists could be locally utilized at intervals and for prolonged effect?

Jacobson: Well, they have been used in long-term experiments on animals, and you can produce megaloblastic changes in the bone marrow with this material, for instance, provided you give it very carefully over a long period In some leukemic children, of course, we also see [illegible] There is one very interestir[illegible] has filmed at the Strangev[illegible] which enter into prophase [illegible] chick embryo fibroblasts—and then proceed into

cells ever went into telophase because they were accumulating or piling up in metaphase.

Levin: I should think this work by Hertz would fit in very beautifully with what has been reported here today, because in a way estrogen is a growth stimulator. If one inhibits growths by means of these folic acid inhibitors then one inhibits also the growth stimulating effects of estrogen.

Engle: Yes. I think it is a perfect correlation. Estrogen is the growth hormone of the genital tract, and it promotes its effect on the epithelium in the genital tract through mitosis of the epithelium. That we know. And with this other suggestion, that an estrogen effect is mediated by the use of folic acid and perhaps some other enzyme system, I think it fits your story perfectly.

Cowdry: I should like to ask a question somewhat similar to yours, Dr. Engle, when you attempted to get information about the sequence of events in spermatogenesis. You have been studying the bone marrow, and you have the primitive cells as well as the erythroblasts and normoblasts. Is there a progressive difference as you pass from the primitive cell to the normoblast? In the materials present in the metaphase, is there a deprivation gradient?

Jacobson: I can't answer that question completely for the following reason: the basophilic erythroblast, the early erythroblast, still shows quite clearly the same distribution of ribonucleoprotein on the chromosomes. Some of the material comes off while the two sets of chromosomes move apart, provided you haven't lost any of this material in the process of treatment. But I don't know whether, for instance, an early polychromatic erythroblast, which would undergo its last division, might not have lost this ribonucleoprotein altogether and thus come to an end of its mitotic life and begin to differentiate into red cells.

Cowdry: Your concept is an interesting one. I was wondering, perhaps, whether there would be differences in the granular leucocytic series of the bone marrow

Jacobson: Yes. I again have to make this reservation In the myeloblast, which can go on dividing, you have the same metaphase and anaphase distribution of ribonucleoprotein, but in the myelocyte, it is very difficult to see ribonucleoprotein on the chromosomes, and I am not sure, when I don't see it, whether I have lost it or whether it has never been there, but it is present in neighboring cells I think this is something which really requires full attention, to decide whether the end of the mitotic life of the cell is in any way connected with the occurrence of ribonucleoprotein on the metaphase chromosomes, and the shedding of ribonucleoprotein during the anaphase, or whether when

this mechanism is exhausted the cell stops dividing.

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metaphase If you are lucky, you can see the same cell reconstructing eventually a resting nucleus again, which is larger than usual. In other words, the cell was capable of altering the normal mitotic process, having advanced first from prophase into metaphase and then instead of dividing, re-forming a resting cell. Now, this sounds so surprising that Dr. Hughes took actual films of it, and I think he showed one of them in New Haven at the International Congress of Cell Biology.

Heilbrunn: That is the same thing that happens with culture cells, isn't it?

Jacobson: Yes, the reconstruction of the resting cell nucleus is very interesting, of course, because a larger cell may result from blocking the availibility of folic acid, and it is obvious that one might think of similar changes occurring when we talk of a megaloblastic bone marrow.

Hamilton: With regard to local effects, is there any possibility of topical administration of aminopterin to avoid some of the general body disturbances? Can it be used locally on the skin?

Jacobson: Well, I don't know whether anybody has done it, but the growth of the hair follicle is affected by folic acid antagonists, and you can produce temporary loss of hair in leukemic children and patients under treatment with aminopterin.

Hamilton: But, again, this is a systemic effect. I am just wondering if it would work through the skin.

Jacobson Oh, yes You can apply it locally. It is very water-soluble, and there is no reason why one shouldn't make it up in a paste. The skin actually has quite a content of folic acid It needs folic acid for its growth, so aminopterin can be used. There is no reason why it shouldn't be, but I don't know of any experiment where it has been used on hair

Levin: Wouldn't you get the effect that you are thinking of on the skin as well as on the hair?

Engle: Don't forget, you might produce changes in the intestinal mucosa, too. Dr. Jacobson, there is one further question I am still concerned about these cells that are interrupted in metaphase and do not complete division Under the circumstances when those metaphases did go on and complete, *in the in toto treated mouse, was an increased* number of abnormal cells produced? Did you begin to get heterochromotosis and phenomena like that?

Jacobson: I haven't seen heterochromosomes, but abnormal cells certainly are produced; for instance, *cells with large nuclei and a large* amount of cytoplasm. I have a suspicion that those cells were actually cells that proceeded into metaphase and then reverted into a resting

stage, but I can't say that, of course, from the histological section I only interpret it that way from Dr. Hughes' observations. The only chromosomal abnormality I have noticed, apart from the clumping of metaphase chromosomes, was occasional aberrent chromosomes which slipped away from the metaphase plate and stuck in the center.

Heilbrunn: I would like to bring up another thing that might have some relation to this We have been interested in the effect of heparin and heparin-like substances on mitosis. Now, heparin is ordinarily a stronger acid than nucleic acid, and it has been shown recently by Wilbur and Anderson that if you treat nucleoproteins with heparin, the nucleic acid is freed Also, very recently, one of my students told me that in the egg of the clam, he was able to detect metachromatic staining substance in the nucleolus Heparin, of course, is metachromatic. Now, there may be a relation between heparin, nucleic acid, and protein Both heparin and nucleic acid compete for the same basic proteins We have been very much interested in the effect of heparin on the stages of mitosis coincident with the appearance of the spindle. This is a rather vague statement, but perhaps in another year or two, we will know more about it

Jacobson: That is certainly very interesting, and there is one other point which I should like to mention When you talk of the inherited material which one cell carries on to the daughter cells by this mechanism of mitosis, we think of desoxyribonucleoproteins But the protein moiety of the desoxyribonucleoprotein is not a very interesting one. It has a rather monotonous amino acid composition, like histones and protamines Relatively few, primarily basic, amino acids are involved in it, whereas the ribonucleoprotein molecule contains all amino acids, as far as we know, and the ribonucleoprotein would, as such, be very much more suited as material to transfer inherited qualities from one cell to the other, as it has the means of expressing a much greater variation in chemical structure.

This is, of course, just a thought The possible variations of the desoxyribonucleoprotein molecule are more restricted and remind me of a situation in which I should have to write a novel with only about fifteen letters of the alphabet available It would be difficult to do. But if you have all the twenty-six letters, you can express really any and every thought you have From that point of view, the blue ribonucleoprotein appeals to me as something which means more

Cowdry I think that if you were to use the Giemsa stain without the May-Grunwald, the pictures would be even clearer

Jacobson It would be clearer for the blue material, yes.

Cowdry But not for the red?

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Engle: Did you apply the May-Grunwald-Giemsa stain to a film of your tissue culture, as living cells, as you would bone marrow cells?

Jacobson: No. The technique was extremely simple, and all you do is take the whole culture as it is, and place it in absolute methyl alcohol for five to ten minutes. Then you lift it out of that and put it into May-Grunwald for ten minutes and lift it out of that and put it into diluted Giemsa for twenty minutes, and you wash it quickly in glass distilled water followed by acetone, acetone-xylene, clear in xylene, and mount It is so simple a procedure that at the tissue culture conference in Cooperstown, they taught it to the beginners of tissue culture work as a so-called short method.

Oliver: Is the method described in the literature?

Jacobson: Well, everybody who attended this congress in Cooperstown could use it The dyes are readily available as it is an established method for the staining of blood films. There are little technical tricks, such as the ones I just mentioned.

Levin: Which one picks up, yes.

Jacobson: Yes, in doing it But I would be delighted to give you all the details if you wish to have them.

Goldzieher: What fixation is used?

Jacobson: Methanol.

Goldzieher: Another method of fixation wouldn't do, for instance, for sections?

Jacobson: The methanol, which, as a histologist, one always considered to be a most dreadful fixative, turns out to be, for small pieces of tissue, and provided you use absolute methanol, one of the best fixatives. If you compare the chromonemata structure of the resting nucleus under phase contrast microscopy, in the living cell, with the methanol fixed cell, there is little difference between them Other standard histological fixatives which are used for larger bits of tissue are not suitable. I think the whole point is that you need to get as rapid a penetration and dehydration of the nucleoproteins as possible. The methanol seems best for small amounts of tissue

Goldzieher: I was asking this question in reference to material that one would like to stain with this method after it had been fixed already in a different way, for instance, in Bouin's solution. Would that be suitable?

Jacobson: Well, no, due to its acid content, it would dissolve out practically all nucleoprotein from the chromosomes, and this labile interchromosomal nucleoprotein would have disappeared practically completely in Bouin's solution.

Oliver: We have had difficulty staining ribonucleic acid which we know is in the rodlets of the kidney cells in chromicized material. When we fixed the tissue with Giemsa mixture we found they stained quite nicely. The chromicized lipid in the rodlets in some way obscured or occluded the reaction, apparently.

Jacobson: Yes, I have seen it quite nicely preserved in some fixed material The method also works very nicely on smears I have done smears from the cut surface of the kidney and liver, and then you can see beautifully the distribution of the ribonucleoprotein

Oliver. Yes, but the smear method disrupts the pattern of the rods, in which we are particularly interested

Jacobson: Oh, yes, it does.

Steele: May I ask a perhaps irrelevant question? The article in *Science* on tissue cultures, I think it was a chicken heart, from which endothelial cells and fibroblasts had grown, showed that the addition of desoxycorticosterone stopped the growth of the fibrous-tissue cells to a much greater degree than it did those of the endothelial cells They were rather nice results Do you know what desoxycorticosterone does to the rate of mitosis in general? What does it do to the growth of cells?

Jacobson: I did some work on that question in Dr Farber's department in the Children's Hospital in Boston, where we had a number of cases of acute leukemia under treatment with ACTH or cortisone. The bone marrows from these patients were very interesting Again, this has not been published, but cortisone and ACTH in the whole patient seem to depress the mitosis in all stages among the leukemic cells. It did not have any effect on the normal erythroblastic cells nor on the normal myeloid cells, but the mitotic rate of leukemic cells changed; I found it at a rate far below anything I had ever seen before, and no phase of mitosis was specifically affected, in other words, the cell was prevented from reaching a stage where it would normally divide again It is a completely different mechanism

Goldzieher Probably just as different as the mechanism of testosterone, which is shown to have a mitogenetic effect on the epidermal cells

Jacobson Oh, yes Well, testosterone induces the cell to reach that stage where—

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Levin May I go back to your interpretation of the reason why a tissue culture cell escaped from the treatment with aminopterin? In this connection, I would first like to ask whether an animal treated

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with one of these folic acid antagonists and at the same time, or shortly thereafter, is given a large amount of folic acid, will this relieve the disability brought about by the antagonist?

Jacobson: Up to a certain level, it will. If you give a large amount of folic acid antagonist, you have to step up the amount of folic acid a hundredfold to overcome it.

Levin: All right. Now, if I heard correctly, you used 1:2000 solution of aminopterin on the tissue culture and you used 2 milligrams in the intact mouse, which I assume to weigh about 20 grams?

Jacobson: One milligram, yes

Levin: Well, that is even less and would calculate out to be a concentration of 1:20,000 in the intact mouse or in actuality only one-tenth as concentrated as the solution you put on the tissue culture. I would think that the intact mouse, even though some cells may not be able to deaminate the material, would be able to deaminate a certain amount of this in cells which are capable thereof, and then overcome the effect of the remainder with its supplies of folic acid itself which the mouse presumably still has. So I still wonder why the intact mouse could not overcome the antagonist as well as did the tissue culture.

Jacobson: Well, the reason is that the connective-tissue cells do not show any lesion at all under those circumstances in the intact animal. The fibroblasts and fibrocytes and osteoblasts, even in a post-mortem of an animal which was given a lethal dosage, appear quite unaffected. Another thing is—I am worrying about the same problem, too, incidentally—that you can quantitatively overcome your aminopterin with the *Leuconostoc-citrovorum* factor.

Oliver: In tissue culture?

Jacobson: Well, that I can't say In the whole animal.

Levin: That is exactly the point If you can overcome it then I should think the intact animal would be at least as able to overcome this considerably more dilute dose that you administered to it

Jacobson: One has to assume that the aminopterin ties up with a protein, just like folic acid, and then a deaminase might not be able to reach the tied-up aminopterin. However, this is purely speculative.

Goldzieher: Would a selective affinity of certain cells for the folic acid antagonist be conceivable, in the sense that they would, so to speak, collect this substance so that more would be coming to them than their proportional share?

Jacobson: I should say one has to assume something of that sort, because some cells grow fast and others do not grow fast, and I do not see why a tissue that doesn't grow very rapidly should take it up at the same rate, unless it actually stores folic acid, like the liver cells

or kidney cells, which contain folic acid. I think one has to assume that it isn't evenly distributed over the whole body. The muscle, for instance, contains relatively little folic acid, and so one can assume that the antagonist also will take a similar distribution

Goldzieher: Selective distribution

Jacobson: Yes. In a way like carbon monoxide which is very firmly bound to hemoglobin and requires three hundred times more oxygen to replace it. A similar mechanism must be thought of for the fixation of aminopterin in the tissue.

Lerm: Except that if the relationship is such as your data certainly seem to imply, we must take into account that the ribonucleoprotein is found all over the body.

Jacobson: Yes, but, of course, there is very little synthesis of ribonucleoprotein in any tissue that isn't actually growing or secreting.

Engle: Are there any further questions, comments, or new points of view? If not, Dr Jacobson, we wish to thank you most heartily and sincerely for your beautiful presentation, which certainly opens up more problems than it solves, as a research arbeit should do And since Dr Fremont-Smith has already given you his thanks and appreciation for your attendance here, it is merely my simple duty to call this Conference adjourned

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with one of these folic acid antagonists and at the same time, or shortly thereafter, is given a large amount of folic acid, will this relieve the disability brought about by the antagonist?

Jacobson: Up to a certain level, it will If you give a large amount of folic acid antagonist, you have to step up the amount of folic acid a hundredfold to overcome it.

Levin: All right. Now, if I heard correctly, you used 1:2000 solution of aminopterin on the tissue culture and you used 2 milligrams in the intact mouse, which I assume to weigh about 20 grams?

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